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THE PRENATAL HISTOGENESIS OF THE HUMAN RENAL PELVIS

BY

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To my parents

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To my parents

PREFACE

This investigation was carried out in the Department of Anatomy of the University of Helsinki, and I am deeply grateful to Professor Niilo Pesonen, M.D., head of the Department, for permitting me the use of the premises and equipment of the Department, thus making this investigation possible. To Assistant Professor Martti J. Mustakallio, M.D., M.A., upon whose suggestion I undertook the study of the prenatal histogenesis of the human renal pelvis, I am greatly indebted for his encouraging interest and unfailing guidance throughout the various phases of the work.

Professor A. Turunen, M.D., head of the First Women's Clinic of the University of Helsinki, Professor U. Uotila, M.D., head of the Department of Forensic Medicine, and Docent E. Ahvenainen, M.D., head of the Pathological Section of the Children's Clinic, have kindly placed at my disposal the necessary study material consisting of the fetuses and of the kidneys of infants and adults. I wish to extend my sincere thanks to them.

My thanks are also due to Mrs. Irene Fristedt, who by her technical skill contributed greatly to the success of the different staining procedures. I also thank Miss Elvi Kaukokallio for translating this report into English.

Helsinki, November 10, 1953.

Antti Paalanen.

CONTENTS

INTRODUCTION

Although birth involves a marked change in the environmental conditions of ontogenesis, it does not constitute the termination of the growth and increase of the tissues, but this continues for some time during extrauterine life. However, this artificial boundary of development is used in the present work, in which the histogenesis of the renal pelvis of the human embryo and fetus has been observed by means of equivalent specimens prepared by given methods of staining.

A. REVIEW OF THE SUBJECT

In studies of the development of the metanephros and the renal pelvis, the *epithelial tissue* has been given the most attention because of its great importance (*e.g.*, Peter 1927, Heidenhain 1937). Although other types of tissue also are significant in the structure and function of the renal pelvis as a whole, the available literature contains only brief references to their development in connection with other subjects.

The epithelium of the efferent ducts of the permanent kidney develops from a rapidly increasing cellular thickening of the dorsomedian wall of the Wolffian duct. Kupffer probably was the first to report, in 1865, this observation in the sheep, in contrast to earlier studies in which it was assumed that the ureter is derived from the wall of the cloaca. His opinion has been confirmed later by several investigators (Weber 1897, Schreiner 1902, Hauch 1903, Felix 1911, Torrey 1943, Ludwig 1947/48). This epithelial anlage is present in a human embryo about 5 mm long and it then grows towards the nephrogenic tissue. These two structural components are thereafter closely interdependent in their development and differentiation (Grünwald 1937, Gruenwald 1939).

In a number of publications in recent years, Ludwig (1944, 1949, 1950/51) has demonstrated that in the metanephric duct of the mouse, and in principle also in that of man, the ureter, renal pelvis and collecting tube are each derived from its own anlage, and that the saccular dilatation, regarded by the earlier investigators (Schreiner, Felix and others) as the primitive renal pelvis, is the first ampulla at the end of the anlage of a common collecting tube. According to Ludwig the division of the metanephric duct occurs by means of branches forming continuously in the growing stem, and the reduction of the collecting tube takes place by eversion, starting basally. These opinions differ from the observations of Heidenhain (1923) and Sonntag (1943) of a dichotomous division and fusing of the collecting tubes (Sonntag).

Contrary to Kupffer's opinion that the epithelium of the renal pelvis anlage of the sheep is arranged in three or four layers, later studies of the human embryo have demonstrated a single layer only (Jánošík 1911, Ludwig 1950/51), which frequently is pseudostratified (Schreiner). In the human fetus this type of epithelium begins to change to transitional epithelium (Felix) at the 70 mm stage.

Diverging from these reports and confirming the observations of Ležava (1934), Zymbal (1936) assumed on the basis of tissue cultures that both the transitional epithelium and the simple columnar epithelium of the collecting tube are derived from the Wolffian duct, but that the latter epithelium is replaced later by a mesodermal epithelium of nephrogenic tissue origin, while the transitional epithelium differentiates from it very distinctly as a skin type epithelium.

Jánošík and Ludwig (1950/51) observed in human embryos of 26 mm length a cell arrangement pointing to the incipient development of *connective tissue* and consisting of a closer packing together of the subepithelial embryonic tissue. Schwab (1939) followed the development of reticular fibres and described processes that grow out from polymorphic cells during the second month of intrauterine life, at the margins of which granules of silver accumulate in metal impregnation. According to Krauspe (1922) the human fetus on attaining the length of 20 cm has a fully formed reticular network in the kidney, scarcely differing from that of the adult. However, I have found in the available literature no

systematic description of the continuous development of the connective tissue as a whole in the renal pelvis and the ureter.

Numerous studies have been made of the *basement membrane* of the kidneys and of other organs, especially in regard to its relationship to the surrounding tissues. Its origin has been a subject of debate and diverging opinions have been advanced. Several investigators have suggested that it is closely related to connective tissue and Merkel (1909) believes it is formed by the condensation of the gelatinous ground substance at the margin between connective and other tissues. It is originally collagenous but may also acquire certain characteristics of elastic tissue, such as were later observed by Hewer (1925) in the embryonic ureter at the end of the second month. In similarity to Hewer, Allara (1950) also points out variations in the occurrence of the basement membrane and its dependence on different functional states. Gersh and Catchpole (1949) call the basement membrane an altered ground substance, which stains very deeply near the surface.

Bairati and Migliardi (1939), on the other hand, consider the basement membranes which appear early in the collecting tubes of the bovine embryo to be products of differentiation of the epithelial cells, although they disappear later and are replaced by connective tissue. Using the electron microscope, Pease and Baker (1950) reached a similar conclusion regarding the maintaining action of the epithelium on the basement membrane in the rat kidney.

Although the arrangement of connective tissue and *muscle fibres* in the wall of the adult renal pelvis and ureter is well known (v. Möllendorff 1930, Krauss 1937, Schneider 1939, Östling 1942, Narath 1940, 1951), the development of muscle tissue has received relatively little attention. In the human embryo of 38 mm length, Jánošík observed the first anlages of the muscular layer in the vicinity of the ureteral epithelium, and according to Felix the muscle tissue of the ureter is already fully developed by the time the fetus has attained a length of 150 mm.

In view of the detailed descriptions that are given in the literature of the close network of *blood vessels* in the postnatal renal pelvis and ureter (v. Möllendorff, Krauss), it is desirable to follow also its development through intrauterine life and to compare the origin of the tissue arrangement in the blood vessels and in the pelvic wall.

According to De Muylder (1945), the *nerve tissue*, also, reveals the twofold origin of the kidney, and he differentiates the cortical nerves from the nerves of the renal pelvis and ureter, which form a plexus in the peripheral and subepithelial connective tissue. In the latter wall area v. Smirnow (1901) described sensory nerve endings and in some places he observed that the nerves have an intraepithelial termination. Knoche (1950, 1951) reported that in the pelvis the renal nerves terminate in the area of connective and muscle tissues and in the blood vessels in the manner of terminal reticulum. A close relationship to the blood vessels was also observed by Epstein and Sotelo (1946) and by De Muylder (1948) in human fetuses aged 5 months. The subject of renal ganglia and nerve cells is still open to discussion, and recently Mitchell (1951), in his studies of fetuses and adults, has considered their presence questionable. Regarding the nature of the autonomic nervous system, Mitchell (1950) holds that the nerves innervating the kidney contain sympathetic and probably also parasympathetic fibres. He has also found both preganglionic and postganglionic fibres in the renal plexus. Structurally most of the intrarenal nerve fibres are held to be unmyelinated.

B. OBJECT OF THE INVESTIGATION

In the investigation here reported, an effort was made to throw further light on the derivation of the types and architecture of the different tissues in the renal pelvis and the ureter, to follow their development during embryonic and fetal life towards structural completion, and to study their consistent preparation for functional requirements and strain. Attention was also paid to phenomena leading to maturity and specialisation of the tissues, with due regard to changes in their stainability, since the conclusions drawn were solely based on observations made from fixed and stained specimens.

C. MATERIAL AND HISTOLOGICAL TECHNIQUE

The composition of the series of human embryos and fetuses examined in this study is seen from table 1. The renal pelvis and the ureter of two children aged six months and two years and of

two adults aged 37 and 50 years have been used as comparative material. The classification of the prenatal series by months has been made according to the table of Mall (1910).

From fetuses No. 6 and 7 only the right, and from fetus No. 34 only the left renal pelvis and ureter were obtained. From the other fetuses of the third to ninth months there were dissected out both renal pelves, frequently with a slight layer of parenchyma, and an adjoining short length of ureter, on which all descriptions of the ureter in this report are based. The embryos of the second month[1] were sectioned intact in series at 10 μ, Nos. 1, 3 and 5 transversely and Nos. 2 and 4 longitudinally.

Embryos No. 1—5 were fixed in Zenker's fluid and fetuses No. 6, 16, 35—37, 40 and 41 in formalin solution (1: 9). Bouin's fluid was used for the remaining preparations. In addition to these, special fixatives were necessary for the staining of nerves in some cases. Parts of the renal pelvis of certain fetuses were placed in these special fixatives, the remaining portions being treated by the usual procedures.

The time of fixation ranged from a few days to several months. The tissues were generally well preserved in the prolonged fixation, although their affinity to stains appeared to be somewhat reduced.

It was the effort to section the paraffin-embedded tissue pieces transversely or longitudinally in series of variable thickness ranging from 3 to 50 μ, with 5 μ as the most common thickness. Thick sections of 20 and 50 μ were used in elastic staining for an increased depth effect in the walls of the renal pelvis and the ureter and blood vessels.

There is a marked difference in the stainability of fetal and adult tissues and many of the combination methods which are excellent for the latter give poor results and weak colour contrasts in immature tissue, even though good staining results are not necessarily a proof that the tissue preparation is true to nature (Zeiger 1949). In addition to age, a number of other factors, such as the species of animal and the situation of the organ in question, influence the staining properties of the tissues. The most appropriate procedure for the purpose should therefore always be sought for in each case.

The various nuclear stains used in this material, which included

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[1] I am greatly indebted to Assistant Professor Martti J. Mustakallio, M.D., M.A., for placing these prepared embryos of the second month at my disposal.

TABLE 1

Second Month				Third Month				Fourth Month				Fifth Month			
No.	Length (cm) CR	Length (cm) CH	Sex	No.	Length (cm) CR	Length (cm) CH	Sex	No.	Length (cm) CR	Length (cm) CH	Sex	No.	Length (cm) CR	Length (cm) CH	Sex
1	1.5			6	3.4	4.0	F	15	8.0	10.5	M	24	13.0	18.5	F
2	1.9			7	3.8	4.6	F	16	8.2	11.0	M	25	13.0	19.0	M
3	1.9			8	3.9	5.0	M	17	8.5	11.5	M	26	14.0	21.0	F
4	2.0			9	5.0	6.5	F	18	9.0	12.0	F	27	15.5	22.0	M
5	2.9	3.3	M	10	5.5	6.8	F	19	9.5	13.5	M	28	16.0	22.5	F
				11	5.5	7.0	F	20	10.0	14.0	M	29	16.3	23.0	M
				12	6.0	8.0	F	21	11.0	15.0	M	30	17.0	24.3	M
				13	7.0	9.0	M	22	11.5	16.0	M	31	17.5	25.0	M
				14	7.0	9.5	F	23	12.0	17.7	M				

hematoxylin (Heidenhain, Regaud, Weigert), acid alizarin blue, fast red and azocarmine G, yielded very good and clear pictures. Of the plasma stains proper, acid fuchsin, picric acid and the azo dye chromotrope 2R proved, in the order listed, very appropriate, especially for differentiation of muscle cells, azocarmine G and phloxin B being less satisfactory. Orange G gave good results in the demonstration of erythrocytes.

The basement membrane was brought out with all the methods employed for connective tissue staining, which will be referred to below, though the sharpness of the membrane varied somewhat.

Stains and silver impregnation were employed for demonstration of the reticular fibres. The latter, used in the modification of Gomori (1937), gave very distinct results irrespective of the method of fixing. Especially in fetal tissue, several of the collagen stains are also adsorbed by reticular fibres. This occurred with, for instance, methyl blue in hydrochloric acid solution according to Lillie's method (1945). Furthermore, after oxidation with periodic acid the reticular fibres appeared to be positive for fuchsin sulfurous acid, but when followed by counterstaining with picromethyl blue according to Lillie (1951) a strong blue colour predominated in all fetal preparations and no indications of allochroism were noted. Interesting, for an unexplained reason variable results, which were comparable in some places to silver impregnated specimens, were

TABLE 1

Sixth Month				Seventh Month				Eighth Month				Ninth Month to Birth			
No.	Length CR (cm)	Length CH (cm)	Sex	No.	Length CR (cm)	Length CH (cm)	Sex	No.	Length CR (cm)	Length CH (cm)	Sex	No.	Length CR (cm)	Length CH (cm)	Sex
32	17.5	26.3	M	36	23.3	34.0	F	38	24.6	38.0	F	40	34.0	48.5	M
33	19.0	27.0	F	37	23.7	35.5	M	39	28.5	43.0	F	41	35.0	50.5	F
34	20.0	30.2	M												
35	22.0	32.5	M												

furthermore obtained with β-methylindol for reticular fibres according to the method of di Maggio (1949). The picture, however, was not clear and the cells frequently were unstained. Furthermore a very long time (over 24 hr.) was required for the action of light to bring out the fibres well in this material.

The staining of collagen fibres was most successful with blue triphenylmethane dyes, *i.e.*, aniline blue and methyl blue, whereas fast green FCF of the same dye group and the nitroso dye naphthol green B were not as satisfactory, possibly owing to the immaturity of the tissue. A similar observation was also made regarding indigo carmine and thiazin red.

In the study of elastic tissue, preference should be given to synthetic orcein (Gurr) (Darrow 1952), since natural orcein co-stained also the background a disturbing red colour, especially in thick sections. Very good specimens were also obtained with resorcin-fuchsin. The new elastic stain aldehyde-fuchsin (Gomori 1950) is interesting from the chemical point and it demonstrates the elastic membranes as well as do the classical methods. The prenatal elastic tissue showed no particular affinity for Congo red (Krajian 1934) and use of this stain was abandoned.

The different tissue types were demonstrated variably by different combination staining methods (Domagk, van Gieson, Heidenhain, Masson, Wallart and Houette, all according to Romeis

1948), and a good all-over picture of the histological structure was obtained by the use of several methods. The new trichrome procedure of Gomori (1950) has the advantage of great facility, but it was not found to possess any further benefits, at least in fetal tissue. By the addition of an elastic stain to the combination method [Mollier 1938, Kornhauser 1943, 1945, hematoxylin-orcein- (resorcin-fuchsin-) picroindigo carmine (Mustakallio)] it was possible also to follow the development of the elastic fibres and their relation to other tissues.

Especially in nerve staining, the optimal reduction of silver salts is greatly dependent on the nature and age of both the organ and the peripheral nerve tissue. The conclusions in this investigation were drawn from observation of different silver impregnations in paraffin sections mounted on slides. Relatively fine results were obtained with the procedures of Holmes (1942, 1943) and Romanes (1946, 1950); the latter has also been recommended by Mitchell (1951) for kidney specimens. The urea-silver nitrate staining of Ungewitter (1951) proved very promising and yielded very sharp and bright cell pictures resembling the results of physical development. My own modification consisting of preimpregnation with buffered silver nitrate solution (1: 10,000) after treatment with chloral hydrate in order to make the specimens basic, followed by reduction and impregnation with silver carbonate solution (prepared according to Jabonero 1952) also proved to be very satisfactory in some cases and confirmed the observations made with other methods. Great caution and critical judgment is required in the interpretation of nerve specimens, as connective tissue fibres frequently have a tendency to co-stain capriciously and variably, and measures taken to reduce this tendency (xylol-glacial acetic acid, pyridine, 5 per cent acetic acid, prolonged treatment with thiosulfate) were of little avail. Although Pearson and O'Neill (1946) have recommended their procedure for embryonic tissues, the co-staining of the connective tissue (possibly due to physical development?) greatly reduced the serviceability of this method.

Although block impregnation is always of uncertain success, the theoretically and practically well founded modification of Weber (1947) was attempted for the kidneys of a few fetuses (Nos. 12, 17, 30 and 40) but with unsatisfactory results. The preparation of frozen sections was also rather laborious, especially in the case of fragile renal tissue in an early stage of development.

In order to demonstrate possible myelin sheaths, tissue pieces from the renal pelvis and the ureter of some fetuses (Nos. 12, 30 and 35) were subjected to vaporising with osmium tetroxide according to Bruesch (1942), and the paraffin-embedded specimens were sectioned at 1 μ.

RESULTS

SECOND INTRAUTERINE MONTH

Embryos No. 1—5,
CR Length 1.5—2.9 cm

The wall of the renal pelvis and the ureter of the human embryo is in the second month composed of epithelial cells and of mesenchyme with very closely packed cells (Fig. 1). No differentiation is as yet observable in this mesenchyme and it is relatively abundant in relation to the amount of mesenchyme in the nephrogenic tissue proper in the kidney. However, a tendency towards the structural arrangement which will be distinctly brought out in the subsequent stages of development is already seen in some of the specimens, the oval mesenchymal cells becoming arranged, in the longitudinal sections, parallel to each other and to the growing epithelial tissue.

Epithelial Cells. — Towards the end of the second month the epithelium covering the renal pelvis, which has a rather large cavity, is in places simple and in other places pseudostratified. The cells are small and cubical, and cytoplasm of a light colour surrounds the round nucleus, which here and there is situated in the central or apical portion of the cell, where the cytoplasm is deeply stained. The arrangement of the cells in a single layer is seen more distinctly in the narrow ureter than in the renal pelvis. The cell margins, however, are not distinctly defined even in the ureter, presumably because of the thickness of the sections. Mitotic figures are frequently observable in the epithelium, especially in the proliferative areas, and in the connective tissue cells (Fig. 2).

Mesenchymal Cells. — These small cells contain a fairly slight amount of cytoplasm, and a large, round or oval nucleus rich in chromonemic filaments almost fills the cell. Already in the youngest embryo in this series (No. 1, CR length 1.5 cm) the cells are closely

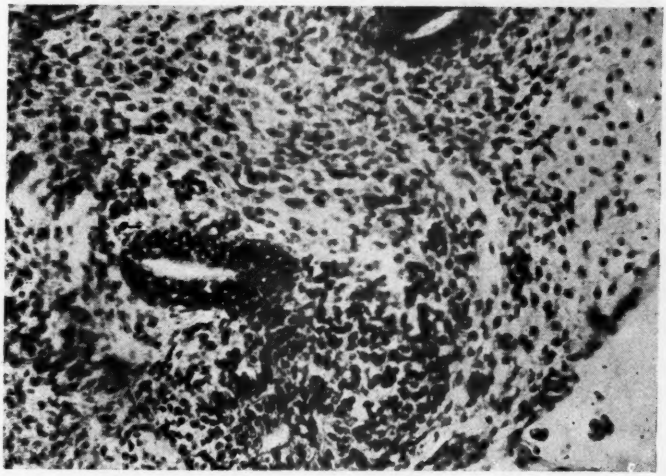

Fig. 1. — Embryo No. 1, CR length 1.5 cm. Cross-section of the left renal pelvis. Hematoxylin-resorcin-fuchsin-picroindigo carmine. 10 μ. × 280.

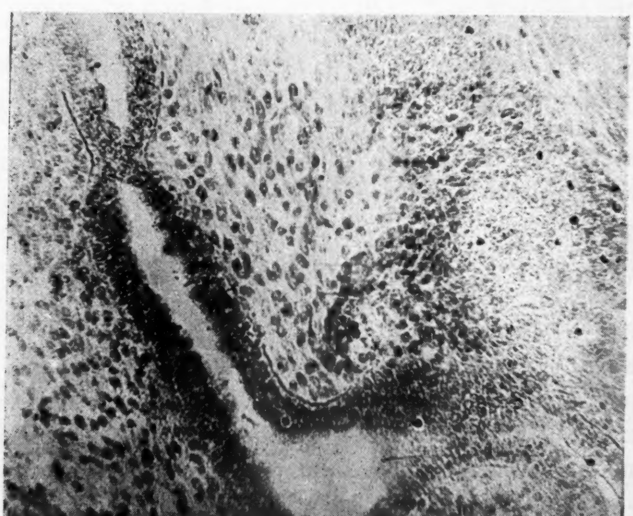

Fig. 2. — Embryo No. 2, CR length 1.9 cm. Longitudinal section of left renal pelvis. Hematoxylin-orcein-picroindigo carmine. 10 μ. × 280. Basement membrane distinctly discernible in some places. Mitotic figures are visible in the epithelium.

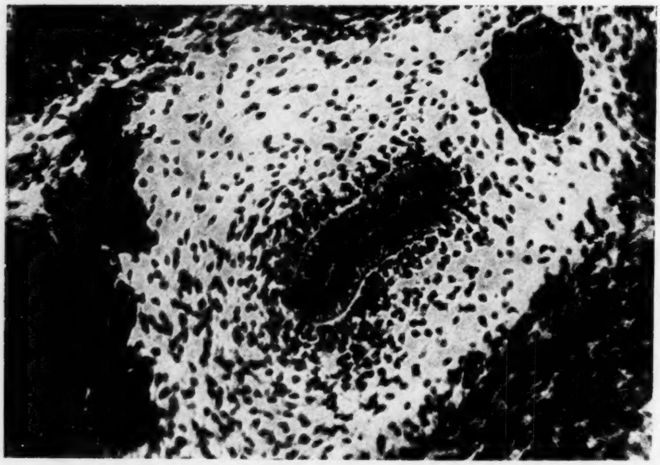

Fig. 3. — Embryo No. 4, CR length 2.0 cm. Longitudinal section of the left renal pelvis. Hematoxylin-orcein-picroindigo carmine. 10 μ. × 280. Accumulation of mesenchymal cells around the basement membrane.

accumulated in the immediate vicinity of the basement membrane of the ureter into a zone consisting of several cell layers which in the cross-section are concentric. A similar formation in the wall of the renal pelvis does not commence until in the end of the second month (Fig. 3).

Basement Membrane. — In collagen staining the basement membrane, which will be more closely examined in the third month, already appears in some places as a sharp boundary between the epithelium and the connective tissue, and in other places, especially in the areas of epithelial proliferation, it disappears, thus closely following the active events of growth.

Other Tissues. — With the exception of connective tissue fibres, which are situated between, and closely adjoining, the mesenchymal cells and which stain a bluish colour with indigo carmine, the combination staining employed did not bring out other mesodermal tissues in the renal pelvis or ureter.

Blood Vessels. — The few fine, small capillary vessels in the wall are restricted chiefly to the dense layer of mesenchymal cells.

THIRD INTRAUTERINE MONTH

Fetuses No. 6—14,
CH Length 4.0—9.5 cm

As the various structural parts of the renal pelvis and the ureter become more defined at this stage of fetal life, the functional and architectonic development and adaptation of the tissues can be observed very distinctly. The epithelium, seen as a continuous band, appears to occupy a central position in the growth of the various tissues, which occurs both independently and in close interrelation with their environment. With an increase in the epithelium, the loose mesenchymal tissue becomes subject to the influence of local, radially moving and of tangential, peripherally moving forces of tension, which, in part at least, may direct the origin and formation of the cell and fibre arrangements. Active development takes place in the mesenchyme in the immediate vicinity of the epithelium, whereas the surrounding tissue maintains throughout this time its large intercellular spaces, in which some cells also may move freely. Thus the cells and fibres are most closely set subepithelially and become more sparse towards the periphery, sometimes very abruptly. These spiral fibres give rise in the third month to the morphological and functional base of the wall structure, which is well demonstrable in the ureter since the latter is histologically more distinct and more dense than the renal pelvis.

Epithelial Cells. — The cells in the epithelial lining of the large round cavity of the renal pelvis of the youngest of the examined fetuses of the third month (No. 6, CH length 4.0 cm) are low, cubical and usually in one layer and have a large nucleus which almost completely fills the cell. Along with this form there are, at the ramifications of the calyces and collecting tubes, layers of epithelial folds produced by cell proliferation. In fetus No. 8 (CH length 5.0 cm), folds of epithelial cells are seen, usually two layers deep, which project from the wall inwards into the cavity and terminate freely (Fig. 4). In addition to these formations, Ludwig (1950/51) has observed actual epithelial membranes at the places of branching to impede the flow of fetal urine. Like Ludwig, I never found the basement membrane to continue into the area of epithelial proliferation. It would seem that epithelial growth at this stage is very

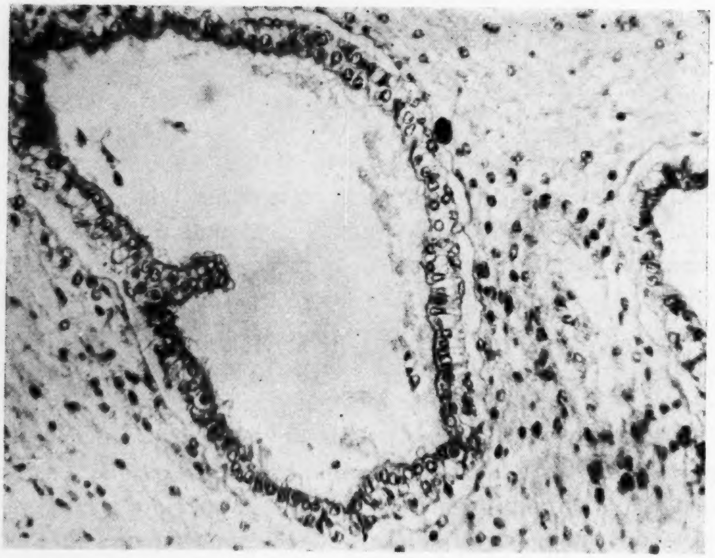

Fig. 4. — Fetus No. 8, CH length 5.0 cm. Cross-section of the left renal pelvis. Kornhauser's staining. 3 μ. $\times$ 280. A group of epithelial cells, two layers deep, projects into the cavity. The basement membrane does not extend to the fold.

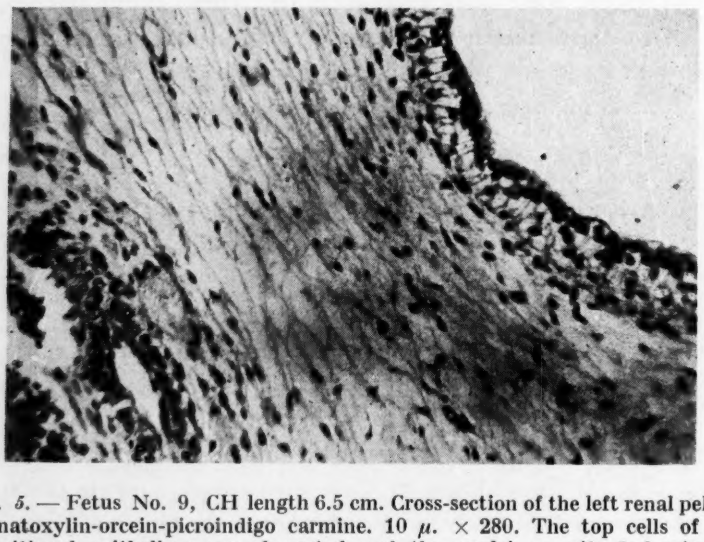

Fig. 5. — Fetus No. 9, CH length 6.5 cm. Cross-section of the left renal pelvis. Hematoxylin-orcein-picroindigo carmine. 10 μ. $\times$ 280. The top cells of the transitional epithelium are elongated and the nuclei are situated apically, where the cytoplasm stains deeply. Cubical cells adjoin the basement membrane. Mesenchymal cells and connective tissue fibres are seen in the wall.

active and that a lack of space forces the epithelium into folds. Gradually these cell groups disappear and are not seen in the later stages of development of the always relatively large renal pelvis.

The epithelium of the collecting tubes has a single layer of large, dark and tall cylindrical cells, in which the nucleus is oval or rectangular, probably because of crowding of the cells.

The boundary between the epithelial proliferation and the low, cubical epithelium is indefinite, and epithelium of a single layer and of several layers may be seen in the wall side by side. This may already be an intimation of its development towards the final, definitive form. The epithelial cells of the renal pelvis then grow quite rapidly in length and in many places arrange themselves into two layers (fetuses No. 9—14, CH lengths 6.5—9.5 cm), corresponding to the basic arrangement of transitional epithelium (Fig. 5). The form of the cells in layers varies from round to elongated, but the cells in single layers are almost without exception very tall and cylindrical. The nucleus, which is well defined, is large and round and contains a moderate number of chromonemic filaments and a nucleolus (or occasionally two nucleoli) situated centrally or slightly excentrically. A relatively large number of mitotic figures may be observed. The nucleus is usually situated apically, especially in the narrow cylindrical cells (Fig. 5). Sometimes no nuclei are visible in the cross-section, sometimes the cells are seen to have two nuclei. The cytoplasm seems to have disappeared to a large extent, but apically it stains rather deeply in the area between the nucleus and the membrane formed on the cell surface (Fig. 5). A seam of cytoplasm is thus formed in the epithelium as a boundary against the contents of the cavity. No actual cover cells are seen. The cell margins are well defined and the cells can be clearly distinguished from each other.

From the point of cell morphology, the epithelium of the renal pelvis has many features in common with that of the thin ureter, although in the later stages of development of the latter the predominant epithelial form is one in which the cells are arranged in two or more layers, distinctly visible, for instance, in the cross-sections (Fig. 6).

Mesenchymal Cells. — The different phases of growth of the developing epithelial band are reflected also in the movement, position and form of the surrounding mesenchymal cells. As also

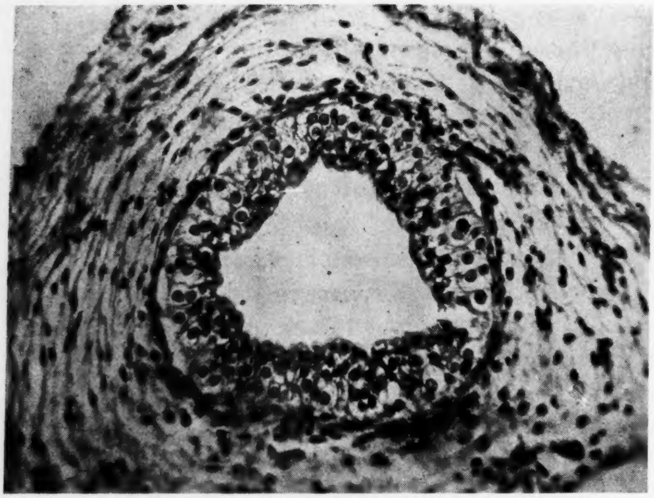

Fig. 6. — Fetus No. 11, CH length 7.0 cm. Cross-section of the right ureter. Hematoxylin-orcein-picroindigo carmine. 10 μ. $\times$ 280. Cells of the transitional epithelium are disposed in two or more layers. Slender muscle fibres form a close ring around the basement membrane.

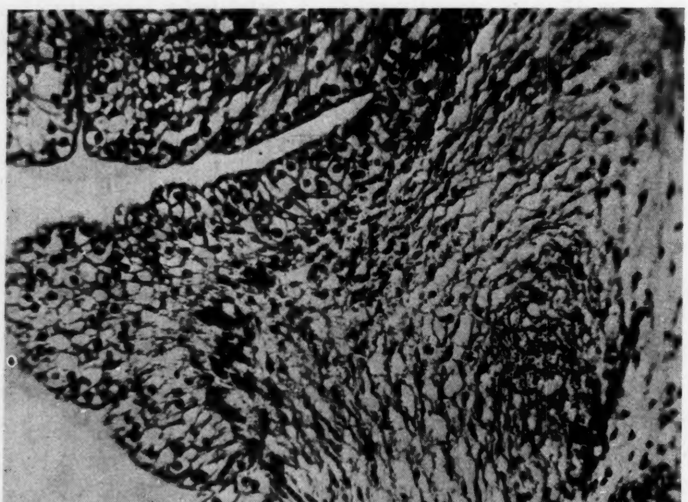

Fig. 7. — Fetus No. 12, CH length 8.0 cm. Longitudinal section of the left renal pelvis. Masson's staining. 5 μ. $\times$ 280. Junction of a developing calyx and the pelvis. A large number of oval mesenchymal cells are seen near the area of epithelial proliferation.

was the case at the end of the preceding month, the number of cells in all the specimens is greater near the epithelium than in the peripheral embryonic connective tissue. In the areas of the epithelial proliferation described above, there is in the renal pelvis marked proliferation and growth of mesenchymal cells. Here these cells, which have become large and elongated, have a tendency to form thick pillars at right angles to the epithelium (Fig. 7), presumably in order to be able later to become evenly dispersed in the wall of the renal pelvis, calyces and collecting tubes.

Most of the mesenchymal cells are small and round, but cells with stellate processes, or oval, flat and large cell types are not rare even in the vicinity of resting epithelium. The nucleus, which is large in proportion to the size of the cell and contains abundant chromonemic filaments, leaves space around itself for only a narrow, pale layer of cytoplasm, thus determining the cell form. The plasma membranes are not as distinctly defined as in the epithelial tissue.

Basement Membrane. — The basement membrane can be observed clearly, although in a variety of forms, in the collecting tubes, renal pelvis and ureter of every specimen. It is distinctly discernible, for instance, with stains of different types (*e.g.*, collagen and elastic stains), but it is delineated particularly clearly against the light background in silver impregnation (Fig. 8). In this staining it is possible to observe that the basement membrane is in some parts composed of numerous slender fibres which in the section appear short and which become attached to each other and form a very close network between the epithelium and the connective tissue. Thus the term »basement membrane», which implies continuity of structure, is somewhat misleading. In the adult, Krauss has replaced it by the term »boundary layer», which seems more appropriate and which may also be applicable to the prenatal substance here and there. These interlaced fibres appear to encircle the epithelium spirally, their angle of elevation varying somewhat according to the shape of the cavity, especially in the wall of the renal pelvis. In the ureter the arrangement of fibres is fairly regular, and for instance in the longitudinal sections the fibres follow this spiral direction at moderately great angles of elevation.

The basement membrane also follows the variations in the epithelial tissue and thus at the sites of epithelial cell proliferation

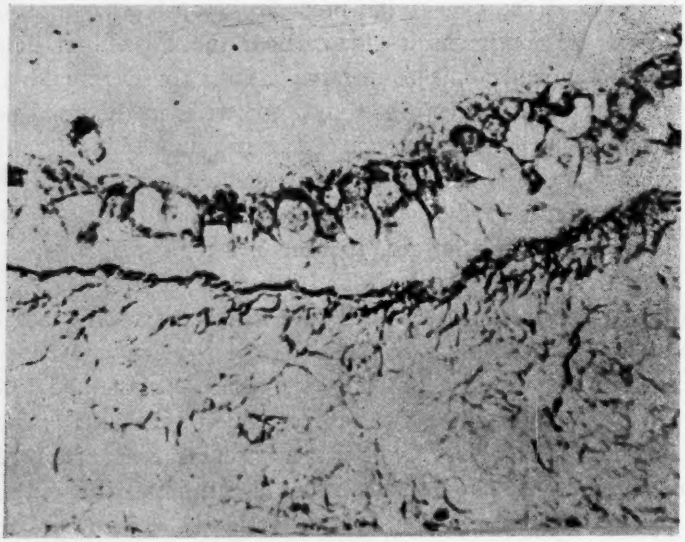

Fig. 8. — Fetus No. 9, CH length 6.5 cm. Longitudinal section of the left renal pelvis. Gomori's silver impregnation. 5 μ. $\times$ 890. Basement membrane at the junction of resting and proliferative epithelium.

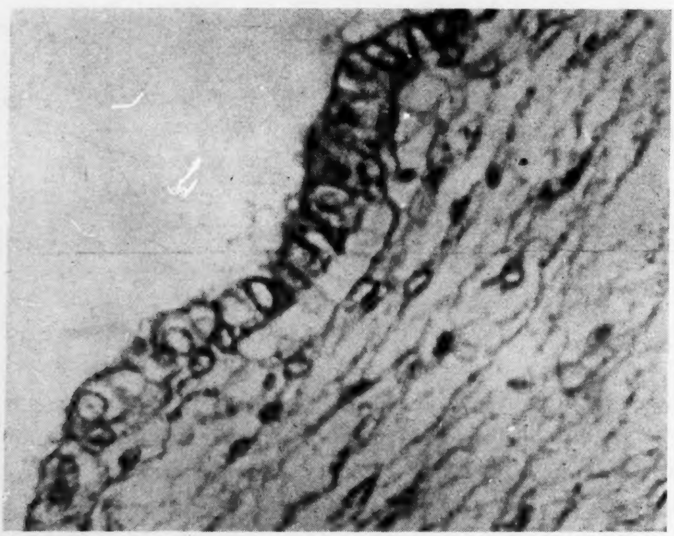

Fig. 9. — Fetus No. 8, CH length 5.0 cm. Cross-section of the left renal pelvis. Gomori's silver impregnation. 3 μ. $\times$ 890. Fibres are visible between the epithelium and the basement membrane.

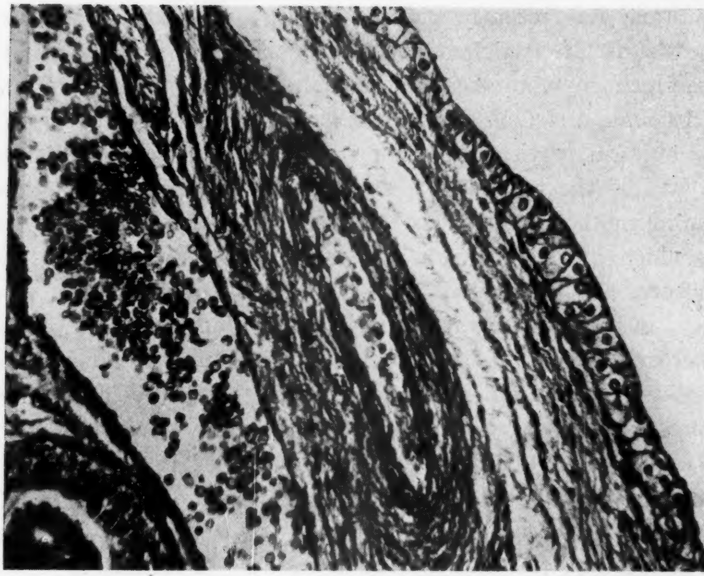

Fig. 10. — Fetus No. 14, CH length 9.5 cm. Slide No. 6/1. Longitudinal section of the right renal pelvis. McManus's technique according to Lillie (1951) without picromethyl blue counterstaining. 5 μ. $\times$ 280. Positive staining reaction in the reticular fibres, basement membrane and elastic tissue of the vessels. Accumulation of fibres is greatest near the basement membrane.

in the renal pelvis the continuity of this membrane disappears (Heidenhain 1937) because of the increase in size of the interfibrous spaces, and the otherwise sharp boundary between the epithelium and the connective tissue becomes indistinct (Fig. 8). At the same time a very marked proliferation and a tendency to parallelism is seen in the adjacent connective tissue fibres.

In preparations in which intact epithelium has become detached from its base, the basement membrane remains in most cases attached to the connective tissue, and in these cases there may occasionally be seen, between the basement membrane and the epithelium, fibres resembling the finest reticular fibres, frequently directed from the former to the plasma membrane of the epithelial cells (Fig. 9). In view of this finding and of the above described observations concerning stainability, connective tissue plays an important part in the development of the basement membrane. Indeed, the congruity of the ground substance of connective tissue, fibroblasts, reticular fibres, collagenous fibres and basement

membrane has recently gained frequent support. Nevertheless, some degree of participation of the epithelium in the formation of basement membrane cannot be excluded with absolute certainty.

Reticular and Collagenous Fibres. — Fibres that appear to be very uniform participate in the formation of connective tissue in the wall of the renal pelvis and the ureter in the third month. Staining methods of various types reveal fibres that vary in tone but which are very much similar in type as to morphological structure, and the network formed by these fibres. The fibres, which, in reducing nitrate of silver, turn dark, take a deep blue colour in collagen staining, especially when methyl blue is used in a highly acid solution. They are also brought out by the technique of McManus, which was tested in a few specimens (Fig. 10). A comparison between these three staining methods in the same renal pelvis showed no marked differences in the number of fibres (Figs. 11 and 12). At this stage of development, therefore, the reticular and collagenous fibres cannot be definitely differentiated.

Early in this month, the fibres are very sparsely and rather evenly distributed in the wall, but after a short time they are seen to accumulate in the vicinity of the epithelium, following the principles of development described above. Alfejew (1926) observed a similar behaviour of reticular fibres towards ento- and ectodermal derivatives. Towards the end of the third month the depth and density of this fibre layer increase and the boundary against the loose peripheral tissue of the sinus becomes accentuated.

The influence of the proliferative epithelial tissue is clearly seen also in the arrangement of reticular and collagenous fibres. Within the sphere of influence of the marked cell proliferation, the number of fibres increases greatly and they become thicker and take a more intense stain. At the same time they have a tendency to become parallel with each other and vertical to the epithelium, thus distinguishing themselves from other structures in the wall (Fig. 13). Later, when the mitotic activity decreases, the fibres so formed presumably become distributed to strengthen the developing tissue.

With age, the number of fibres gradually increases throughout and the fibres, originally pale and thin, become darker and stronger, especially in the vicinity of the basement membrane, all the transitional forms, however, being preserved. The architecture

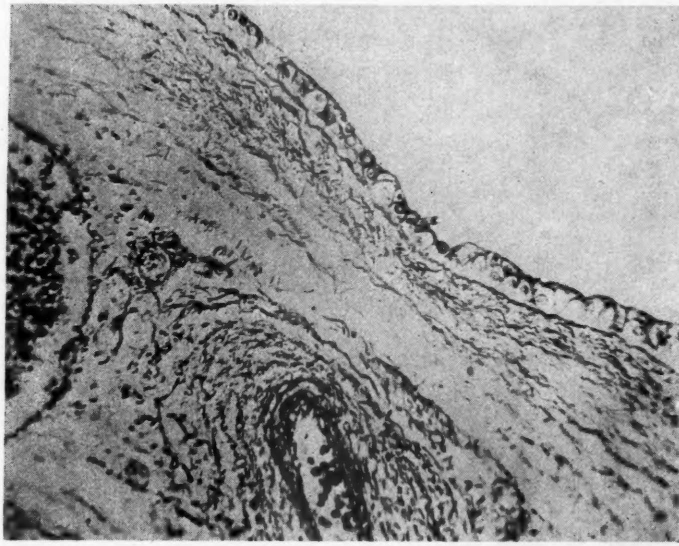

Fig. 11. — Fetus No. 14, CH length 9.5 cm. Slide No. 6/2. Longitudinal section of the right renal pelvis. Gomori's silver impregnation. 5 μ. $\times$ 280. Reticular fibres in the walls of the renal pelvis, artery and vein, and basement membrane and elastic tissue of the vessels are demonstrated. Cf. Fig. 10.

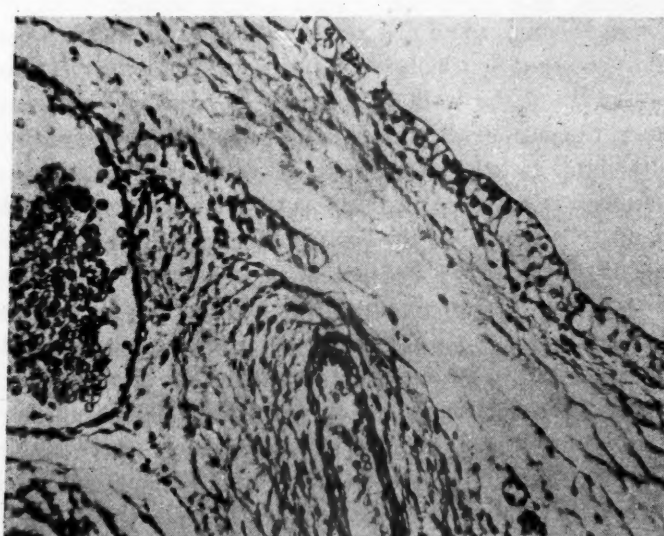

Fig. 12. — Fetus No. 14, CH length 9.5 cm. Slide No. 6/3. Longitudinal section of the right renal pelvis. Lillie's staining (1945). 5 μ. $\times$ 280. Corresponds to Fig. 11. Cf. also Fig. 10 and the text (page 28).

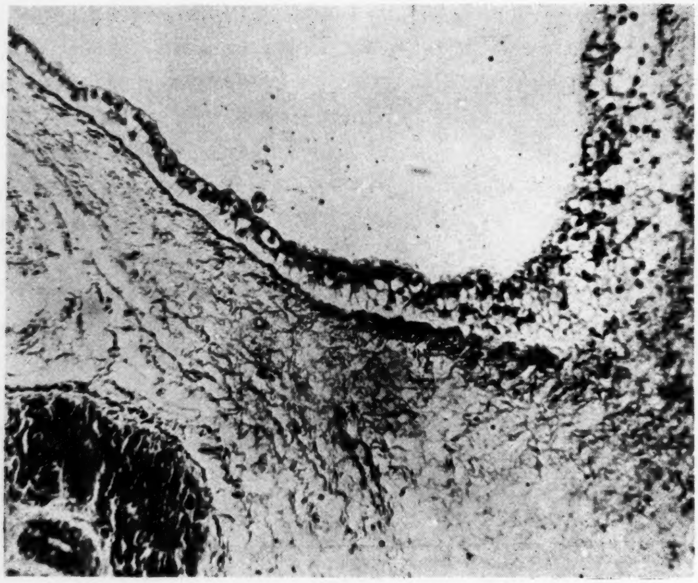

Fig. 13. — Fetus No. 9, CH length 6.5 cm. Longitudinal section of the left renal pelvis. Gomori's silver impregnation. 5 μ. $\times$ 280. Note the effect of the epithelial proliferation on the basement membrane and reticular fibres.

of the wall becomes more distinct as the wavy fibres grow around the cavity in spirals, which have a variable angle of elevation in different parts of the wall. In longitudinal sections, especially of the ureter, the peripheral longitudinally parallel fibres curve towards the epithelium, and the angle of elevation again opens in or beside the basement membrane. In cross-section the concentrically lying fibres and cells dominate the picture. The arrangement is not as clear in the renal pelvis because of variations in form, many of the fibres attached to the basement membrane may lie diagonally, and the spirals may be intertwined into a close network by means of connecting fibres. An architectural structure of the same type was found in collecting tubes by Niessing (1935) and Schwab.

The relationship of reticular and collagenous fibres to mesenchymal cells has been a subject of dispute. Tissue cultures have shown that the fibres are formed indirectly from the ground substance or directly from connective tissue cells, and the latter opinion has gained increasing support among investigators.

A fixed and stained specimen alone does not provide definite

evidence to solve the dispute on the relationship of mesenchymal cells to reticular or collagenous fibres. However, fibres which adsorb indigo carmine and certain triphenylmethane dyes appear to arise from the ends of spindle-shaped cells and to reach out towards processes from other cells. Silver impregnation brings out only black fibres, the mesenchymal cells having possibly been destroyed by the strong solutions. This problem, therefore, still remains obscure.

The fibres do not form branches but, in staining with metals, for instance, they are interlaced in layers at different levels, frequently being thicker and forming bunches of fibres in the vicinity of the basement membrane.

Elastic Fibres. — The morphological and tinctorial properties of the elastic fibres make their examination difficult in the renal pelvis and the ureter at the early fetal stage. The classical staining methods bring out fibres in the wall which partly resemble those described in the preceding chapter and greatly differ from the elastic fibres in the adult renal pelvis. There is, however, no exact time for their appearance. In resorcin-fuchsin staining they are seen in this series in a fetus 4.0 cm in CH length (No. 6), especially in the thick sections, but there does not appear to be an affinity for synthetic orcein before the latter half of the third month. It is also well to remember the possible effect of postmortal reactions (Lansing 1951) on the structure and stainability of the fibres.

Quite early the slender elastic fibres are spread out as a very independent and distinct group which forms an even network in the wall of the renal pelvis and the ureter (Fig. 14). They prove rather resistant against the effects of epithelial proliferation, as compared with the other tissue types described. Nevertheless, in similarity with them the elastic fibres, which become stronger with age, decrease abruptly in number towards the loose peripheric connective tissue.

The individual elastic fibres are slender, uniform in thickness, and coiled in form, and they greatly resemble each other. In thick sections, which increase the effect of depth, their dense network is clearly visible in the oldest fetuses in this month. These separate fibres, which extend from the periphery all the way to the basement membrane, possibly also encircle the cavity in spirals that are attached to the other components of the wall.

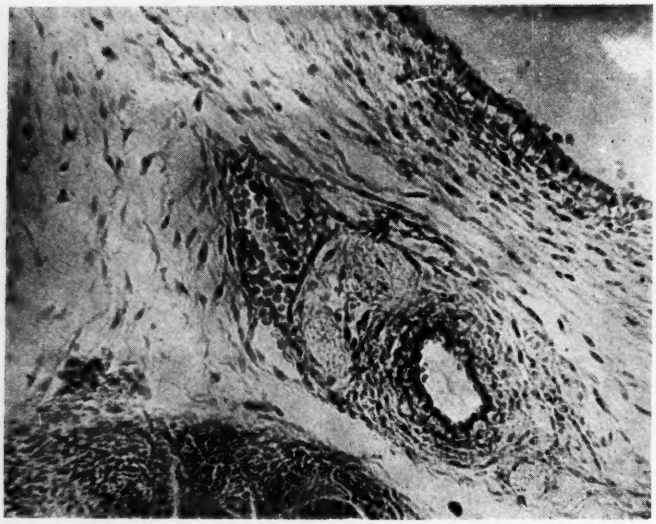

Fig. 14. — Fetus No. 9, CH length 6.5 cm. Longitudinal section of the right renal pelvis. Synthetic orcein. 20 μ. $\times$ 280. The basement membrane and internal elastic membrane of artery are seen in some places. The light-coloured network of a few thin elastic fibres is seen in the wall.

The above mentioned early fibres classified into the elastic tissue remain rather light in colour in the usual elastic staining but become slightly pink after treatment with acid Congo red. Thus there is brought out with the first mentioned staining a marked difference as compared with the adult renal pelvis, in which the fibres stain an intense dark brown colour and are thick, branched and interrupted, and chiefly restricted to the muscular layer.

In the early stages of development the fibrous types of connective tissue are very similar in their characteristics in the renal pelvis and the ureter. The differences, however, are gradually brought out with increasing clarity as time goes on.

Muscle Fibres. — In addition to the protective function of the above described tissues and of their passive elastic action directed towards preservation of the form and structural framework, a description of the development of muscle tissue should take into consideration also its functional adaptation.

The muscle fibres do not appear suddenly and clearly. About the middle of the third month there may at first be seen, near the basement membrane, large cells in which the elliptical nucleus,

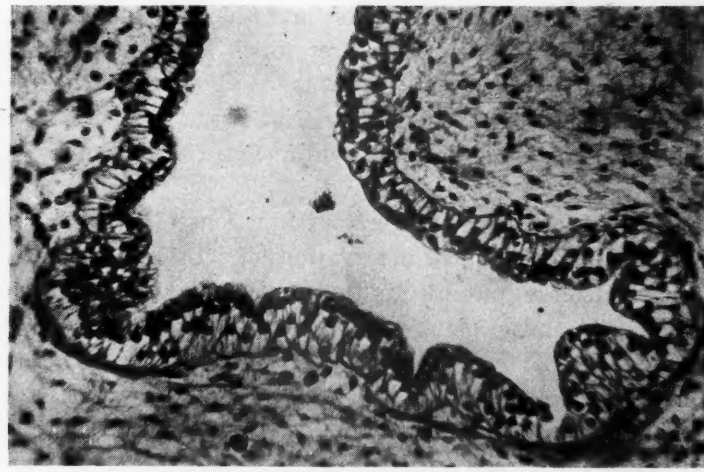

Fig. 15. — Fetus No. 13, CH length 9.0 cm. Cross-section of the left renal pelvis. Hematoxylin-orcein-picroindigo carmine. 10 μ. × 280. Narrow muscle fibres closely adjoin the basement membrane.

rich in chromonemic filaments, is surrounded by a small layer of cytoplasm which poorly adsorbs different plasma stains. These are presumably preliminary forms of muscle cells and their differentiation from mesenchymal cells is frequently difficult. Only towards the end of the month, when the cytoplasm processes reach out towards each other and extend in length and their staining affinity increases, these cells become defined from other tissues as distinct muscle cells. A narrow area filled with connective tissue and capillaries always remains between the cells and the basement membrane.

The ureter is slightly more advanced than the renal pelvis in the development of muscle fibres, since cross-sections of the ureter show adjoining muscle cells that form a narrow layer around the cavity already in a fetus 7.0 cm in CH length (No. 11) (Fig. 6), whereas muscle fibres make their appearance to strengthen the renal pelvis only after the fetus is *c.* 1—2 cm longer (Fig. 15).

Although the muscular tissue is somewhat stronger at the end of this fetal period, only some indications can as yet be seen of the muscle fibre arrangement which later becomes clearer. The large fibres seen in cross-section of the ureter presumably encircle the epithelium in circles or spirals. In the renal pelvis their course

appears to be variable, and sections made at different levels show muscle fibres of varying thickness which are restricted to the wall of the pelvis proper and do not extend forwards to the calyces or fornices. A large number of connective tissue fibres and mesenchymal cells, on the other hand, continue up to the summit of the fornix.

The theory of the early excretory functions of the kidney has been supported by frequent observations of substances physiologic for urine which are contained in the amniotic fluid (*e.g.*, Shaw and Marriott 1949) and of giant glomeruli encountered in central portions of the renal parenchyma (Ludwig 1950/51 and my own specimens). The excretion of fetal urine, which at first is passive, is probably greatly promoted by active contractions of the muscle tissue of the renal pelvis and the ureter, which grows stronger in the end of the third month.

Blood Vessels. — The same structural principle which was seen in the renal pelvis and the ureter is also evident in part in the blood vessels in their walls. The tissues vary greatly, however, according to the type and size of the blood vessels.

A fairly close capillary network, covered with endothelial cells, is situated in the fibrous layer and extends to the basement membrane. This network increases as development progresses. Capillaries cut at different levels in the same section reveal a tortuous course towards the epithelium and a simultaneous decrease in their diametre.

The small arteries and veins which form the origins and terminals of the capillaries are embedded in loose connective tissue. In the first specimens of the third month there are already seen accumulations of oval mesenchymal cells with small nuclei around the lumen, and of reticular and collagenous fibres, both of the latter encircling the lumen as longitudinal spirals. At an early stage the elastic tissue also begins to support the arterial wall, in which there appears at first a large number of longitudinal fine, dark elastic fibres. Already in the renal pelvis of fetuses 5.0 and 6.5 cm long (CH) (Nos. 8 and 9) these fibres aggregate to form distinct elastic membranes (Fig. 14), which thereupon increase in thickness as the development proceeds. The internal elastic membrane which borders upon the endothelial tissue is seen in cross-section as a closely undulating line, and in longitudinal section as a nearly continuous

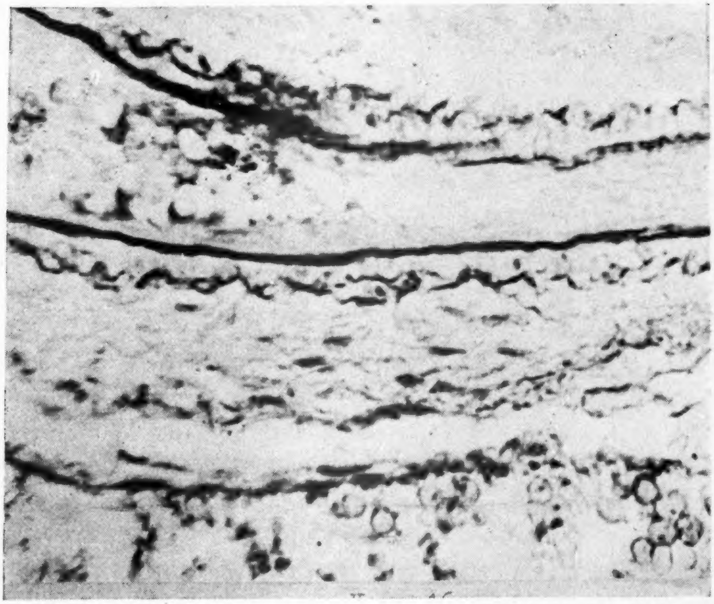

Fig. 16. — Fetus No. 14, CH length 9.5 cm. Longitudinal section of an artery and vein in the left renal pelvis. Resorcin-fuchsin. 5 μ. $\times$ 890. Elastic membranes are clearly demonstrated in the artery. In some places in the vein there is a thin formation suggestive of an elastic membrane.

line (Fig. 16). The wavy structure and the gradual, longitudinal spirals of the folds are seen especially in thick sections made at different levels. The external elastic membrane is also sharply defined, estimated to be about one-half as thick as the internal elastic membrane. It is in many places a fibrous network and is not as dense as the internal elastic membrane, but the structural principle is the same in both membranes. Thin elastic fibres of the tunica media are in cross-section frequently very long and encircle the cavity, but they can also be seen proceeding from the external elastic membrane into the substance of the tunica media (Dürck 1907). The structure of the arteries thus reflects already in the third fetal month its response to the increasing mechanical requirements and strain.

Near the internal elastic membrane are seen a large number of elliptical cells containing large nuclei. In longitudinal sections of the arteries these cells lie transversely and form the anlage of the circular muscle fibres of the tunica media.

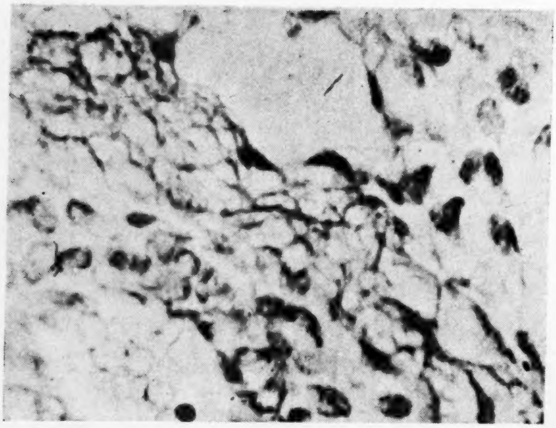

Fig. 17. — Fetus No. 13, CH length 9.0 cm. Longitudinal section of the left renal pelvis. Romanes's staining (1950). 5 μ. $\times$ 890. Nerve fibres in the vicinity of an artery in the pelvic wall.

The walls of the veins are extremely thin and the different connective tissue fibres are intertwined, with indefinite margins. In longitudinal section the reticular and collagenous fibres lie in longitudinal spirals. Elastic fibres also participate in the structure of the veins, in which, at the end of the third month, resorcin-fuchsin brings out here and there a very delicate, narrow and variable formation pointing to the internal elastic membrane.

Nerve Fibres. — The development of the nerve fibres in the renal pelvis and the ureter is closely connected with that of the blood vessels but it nevertheless also follows a more independent course in the loose connective tissue of the wall. In the specimens studied it is possible to observe in the latter half of the third month a few very fine, lightly impregnated nerve fibres, especially near the arteries (Fig. 17). Some of these fibres are also seen in the subepithelial connective tissue, sometimes forming a close network. No distinct intraepithelial terminations are seen, however. Osmium tetroxide vapourising gave negative results in the renal pelvis of a fetus of 8.0 cm CH length (No. 12), which bears out the opinion regarding an unmyelinated structure of nerve fibres. In addition to other connective tissue fibres, some long, dark elastic fibres are seen in the connective tissue surrounding the thick nerves in the periphery of the renal pelvis wall at the end of the third month.

FOURTH INTRAUTERINE MONTH

Fetuses No. 15—23,
CH Length 10.5—17.7 cm

With the fourth month, a phase of tissue strengthening and structural clarification commences in the development of the renal pelvis and the ureter. It thereupon continues as a characteristic feature through the following periods up to birth.

Epithelial Cells. — The epithelial structure described in the preceding chapter is similar in type through the fourth intrauterine month. In the wall of the renal pelvis the cells lie variably in one or two layers and again show marked proliferation at the bifurcations of the collecting tubes and the calyces. As a result, strong bridges of epithelium are formed between the developing calyx and the pelvis. These bridges can be followed in serial sections even in the form of solid bands which often are some tens of μ in thickness before the first connective tissue fibres appear between the epithelia.

In the fetuses in this series the epithelial cells in the ureter appear to be arranged in 3 or 4 layers, the cells in the middle layer being tall and cylindrical and those bordering on the cavity or the basement membrane cubical. The cells are usually larger than in the pelvis. A fearly prominent feature, especially in the latter half of the fourth month, is the fold formation of the epithelium, basement membrane and subepithelial connective tissue in the upper portion of the ureter. A few weeks earlier this fold formation was insignificant, although the thickness of the cell layer was the same in the compared specimens.

Special attention should be drawn to cover cells which appear in the epithelium of the renal pelvis and the ureter about the middle of the fourth month (Fig. 18). In longitudinal section these cover cells are flat and elongated, and their largish, oval nucleus is surrounded by a small layer of cytoplasm, which is denser in the apical section. These cells are present here and there only and in the intervening spaces the usual surface cells of transitional epithelium, containing dark cytoplasm, protrude towards the free cavity.

Mesenchymal Cells. — The localisation of the mesenchymal cells in relation to the epithelium and their reaction to proliferation

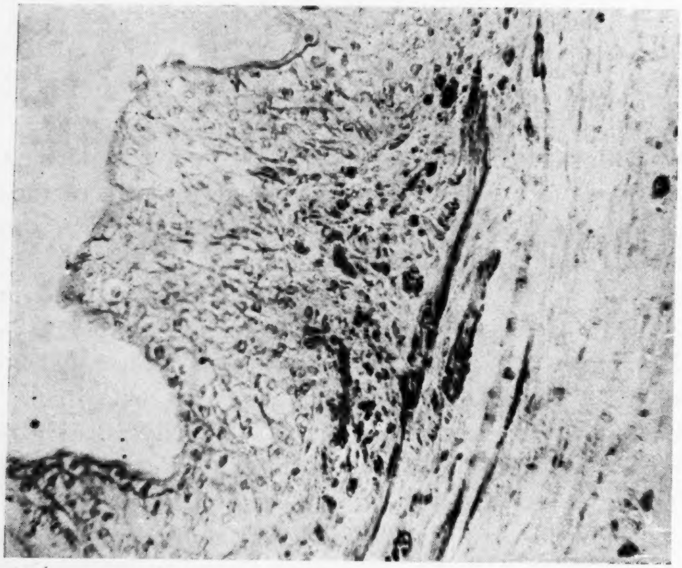

Fig. 18. — Fetus No. 21, CH length 15.0 cm. Cross-section of the left ureter. Hematoxylin-orcein-picroindigo carmine. 10 μ. $\times$ 280. Cover cells are seen here and there. The connective tissue follows the folds of the epithelium. Some fairly strong muscle fibres are evident.

follow entirely the previous line of development, and the thickness and density of the cell layer increases along with other tissue development. Among the mesenchymal cells may also be seen rather large spindle-shaped cells resembling fibroblasts and containing a large amount of pale cytoplasm around an oval nucleus. Thus the mesenchyme is seen to be already transformed to loose connective tissue.

Basement Membrane. — The theory of the fibrous structure of the basement membrane and of its close connection with connective tissue is supported by the finding of closely adjoining fibres in several places and especially at the sites of epithelial cell proliferation. At the same time it nevertheless forms a characteristic dense tissue that differentiates very distinctly from its surroundings.

Reticular and Collagenous Fibres. — Comparisons of the morphological and tinctorial alterations of the connective tissue fibres with the increasing age of the fetus reveals clearly the slow development and specialisation during fetal life. Very little thickening of individual reticular and collagenous fibres and deepening

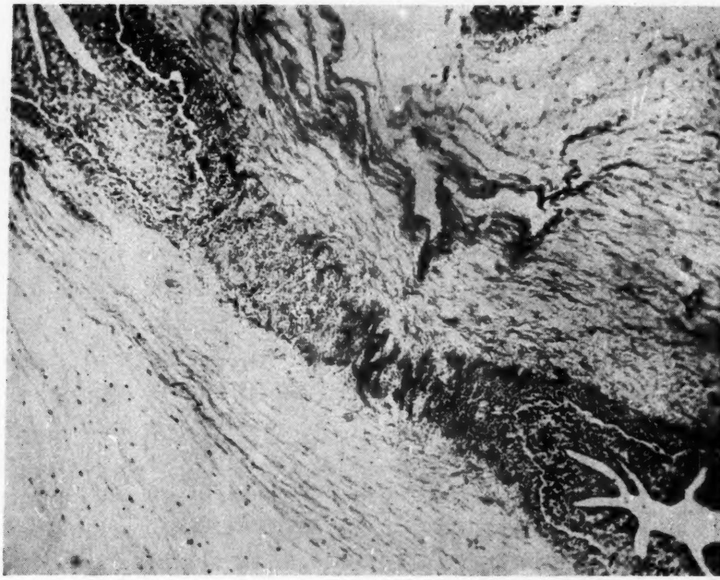

Fig. 19. — Fetus No. 23, CH length 17.7 cm. Longitudinal section of the left ureter. Gomori's silver impregnation. 5 μ. $\times$ 90. The fibrous architecture of the wall is very distinctly brought out.

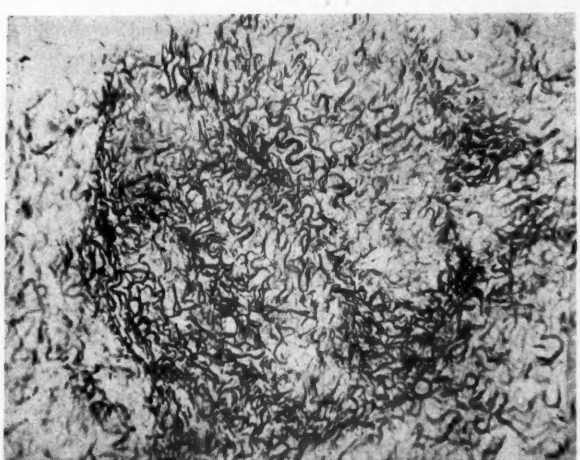

Fig. 20. — Fetus No. 21, CH length 15.0 cm. Longitudinal section of the right ureter. Gomori's silver impregnation. 5 μ. $\times$ 280. Strong fibres are seen in the middle portion of the wall. The fibre groups correspond in site to muscle cells.

of their colour takes place in the fourth month, though the fibrous layer in the wall increases greatly in thickness and the architectural arrangement becomes clearer (Fig. 19).

The chiefly longitudinal and parallel fibres and fibre bundles in the periphery and near the basement membrane are relatively fine but become thicker towards the centre of the wall, where they form, as the angle of elevation decreases, a close network of fibres lying in different directions (Fig. 20). This picture is more clearly defined in the ureter than in the renal pelvis, but in both the fibrous layer ends abruptly against the loose connective tissue.

In several specimens prepared by silver impregnation, and especially in the oldest fetuses of the fourth month, this network is seen to contain groups of long, fine fibres. These differ from their surroundings and exactly correspond in localisation to the muscle cells in the following serial sections stained by the combination method, in which the fibres are brought out by collagen stains. Thus a special thin sheath encloses and strengthens the muscle cells situated in the connective tissue.

Elastic Fibres. — A network that is rather marked but is composed of fibres that nevertheless stain very lightly with the usual elastic stains is spread in the wall with other building materials consisting of connective tissue, especially around muscle cells and capillaries. The morphologically uniform elastic fibres are arranged in spirals around the cavity in the manner of reticular and collagenous fibres, due, at least in part, to tension and functional demands.

In both the ureter and the renal pelvis the elastic fibre layer, which peripherally is well defined, extends up to the basement membrane. The direction of the fibres, which in the ureter is chiefly longitudinal, is in the pelvis variable, due to the form of the organ. In the loose connective tissue there are seen here and there some thick elastic fibres.

Muscle Fibres. — In the first specimens in the series of this month the narrow muscle cells described above are still situated in the immediate vicinity of the basement membrane but stand out here from the surrounding connective tissue cells because of their size and dark cytoplasm. At about the middle of the month and especially thereafter these muscle cells, whose large, oval nucleus has a great abundance of chromonemic filaments, gradually

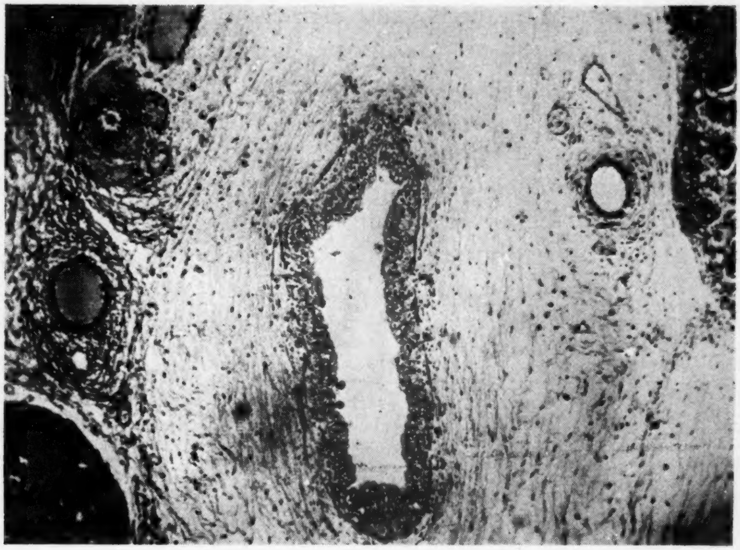

Fig. 21. — Fetus No. 20, CH length 14.0 cm. Longitudinal section of the right renal pelvis. Kornhauser's staining. 5 μ. $\times$ 90. Slender muscle fibres are visible in the vicinity of the basement membrane. Connective tissue fibres are light and arterial elastic membranes in the adventitia strong and dark.

grow long and thick and develop cytoplasmic processes that reach out towards each other to join the cells together. At the same time they recede from the subepithelial layer of connective tissue.

The best picture of the anatomically uniform arrangement of muscle cells in the renal pelvis and the ureter is obtained by following their development. The muscle cells, especially in the ureter, tend to become longitudinal spirals, with the largest angle of elevation in the periphery and near the subepithelial connective tissue. This angle becomes acute where the middle part of the spirals approaches the horizontal plane. Especially these parts, which correspond to the circular musculature, become stronger in the fourth fetal month, but on both sides of them there also are occasional diagonal or longitudinal slender, delicate muscle fibres. Those of the fibres which are adjacent to the subepithelial connective tissue frequently extend deep into the folds of the mucous lining. Because of the small area over which they are distributed, these fibres are more densely situated than those in the periphery (Schneider).

The muscular tissue in the renal pelvis is much weaker and more

indistinct than in the ureter, although the structural principle is the same. The frequently mentioned longitudinal muscle fibres are here situated fairly near the basement membrane (Fig. 21), the intervening layer of connective tissue being very narrow. There is no thickening of the circular muscle fibres that would point to a sphincter muscle between the renal pelvis and the ureter or the calyces. The muscular tissue continues in a weaker form into the walls of the calyces but does not extend to the fornix.

For the muscular tissue the fourth month is, accordingly, a period of rapid maturing and development towards its definitive fibrous architecture.

Blood Vessels. — The different structural components of the walls of the arteries and veins in the loose connective tissue surrounding the renal pelvis and the ureter become more distinct, and they already resemble at this stage postnatal blood vessels.

In the arteries, elongated endothelial cells and a strong internal elastic membrane form the tunica intima, while the tunica media consists of delicate, chiefly circular muscle fibres. The tunica adventitia contains a reticular external elastic membrane of fine, dark elastic fibres, many of which continue to the middle coat. In addition to connective tissue cells the adventitia contains reticular and collagenous fibres of varying thickness. As the external and internal elastic membranes become thicker, their mutual ratio of thickness remains nearly unchanged. However, thin, longitudinal, spiral elastic fibres, which are seen as dots in the cross-sections, now appear outside the external membrane.

The growing venous walls show, in addition to reticular and collagenous fibres, a large number of fine elastic fibres, which frequently have a longitudinal spiral course. In some places there is also a thin internal elastic membrane, which is not present throughout, and some narrow muscle cells among mesenchymal cells.

Nerve Fibres. — The methods of Romanes (1946) and Ungewitter gave very good staining results in a formalin-fixed renal pelvis from a four months old fetus 11.0 cm in CH length (No. 16). The nerve fibres in the subepithelial connective tissue are in this specimen somewhat stronger (Fig. 22) but they still are light in colour and few in number, and are only found fairly near the basement membrane. Adjoining the adventitia of the blood vessels and

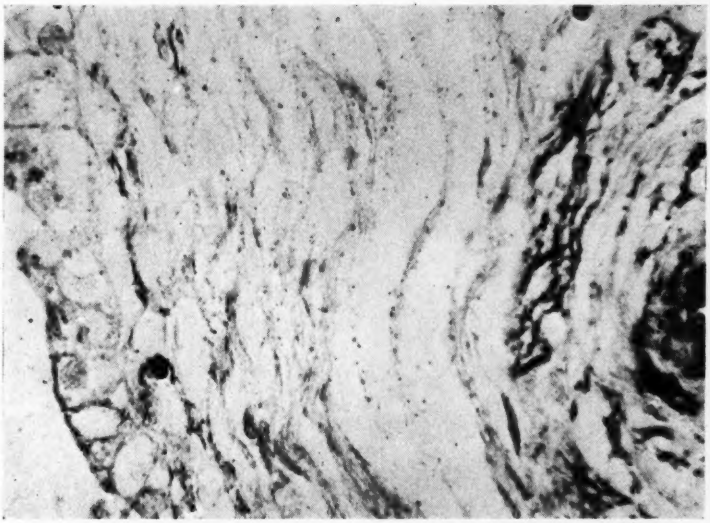

Fig. 22. — Fetus No. 16, CH length 11.0 cm. Cross-section of the left renal pelvis. Romanes's staining (1946). 5 μ. × 890. Nerve fibres are visible in arterial adventitia and in subepithelial connective tissue.

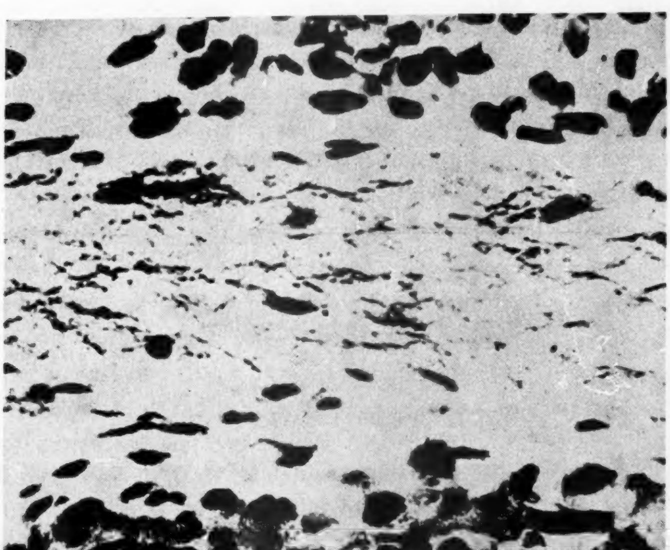

Fig. 23. — Fetus No. 16, CH length 11.0 cm. Cross-section of the left kidney. Ungewitter's staining. 5 μ. × 890. Nerve fibres are seen between an artery and vein in the pelvic wall.

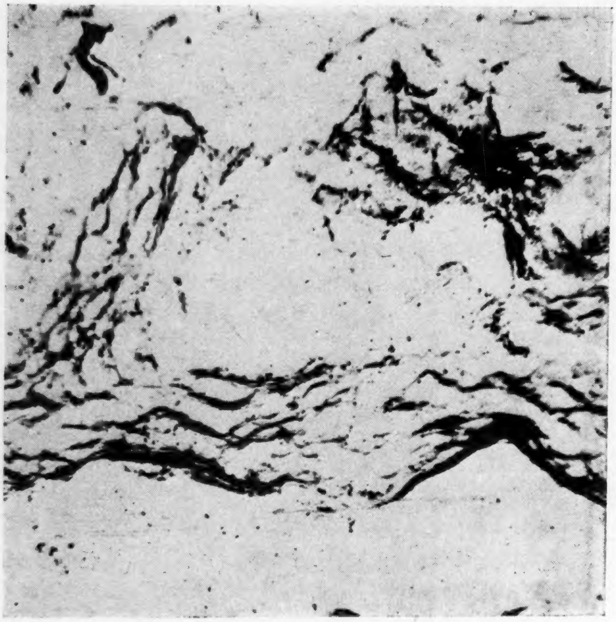

Fig. 24. — Fetus No. 23, CH length 17.7 cm. Longitudinal section of the right renal pelvis. Silver carbonate staining. 5 μ. $\times$ 700. Nerve fibres are seen in periphery of wall, near an artery.

situated sometimes between the artery and the vein, there may also be seen these strong nerve fibres, often in bundles (Fig. 23). This feature was also brought out by the silver carbonate method in the renal pelvis wall of the last fetus of the fourth month (No. 23, 17.7 cm in CH length) (Fig. 24).

FIFTH INTRAUTERINE MONTH

Fetuses No. 24—31,
CH Length 18.5—25.0 cm

At the end of the fifth month of fetal development the difference between the fetal and the full-term renal pelvis and ureter in regard to the structure of the wall has diminished further, especially as concerns the tinctorial and morphological properties of the tissues.

Epithelial Cells. — In the renal pelvis, the cells of the transitional

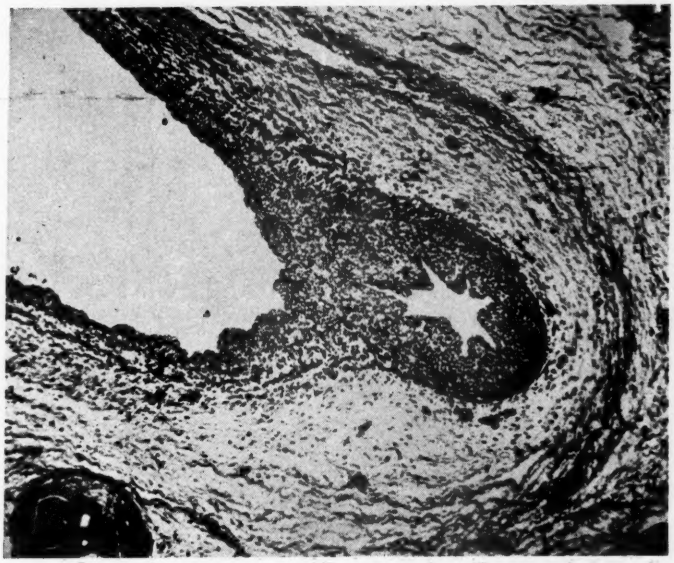

Fig. 25. — Fetus No. 25, CH length 19.0 cm. Diagonal section of the junction of the right renal pelvis and ureter. Mollier's staining. 7 μ. $\times$ 90. The reaction of surrounding tissues is clearly visible.

epithelium, which are light in colour and have large nuclei, lie in two or three layers. When the ureter is contracted the epithelium may be stratified and lie in folds. In many places the epithelium may be covered with flat cover cells, which are seen in but few places in the pelvis.

At the junction of the renal pelvis and the ureter the profuse epithelial proliferation is seen in the cross-sections as a marked fold formation of stratified epithelium, which is not accompanied by the basement membrane and the connective tissue. This observation could be made also in the corresponding specimens in the third and fourth months. At this junction as well as at the bifurcations of the calyces the surrounding tissues react to the most active proliferation in the manner earlier described (Fig. 25).

The fold formation in the ureteral epithelium may thus be due both to cell proliferation (Meyer 1946) and to muscular contraction. In the latter case the subepithelial connective tissue, especially after the mechanical pressure of cell proliferation discontinues, also extends into the folds and the epithelial cells become arranged in three or four layers. The cylindrical cells comprise the majority,

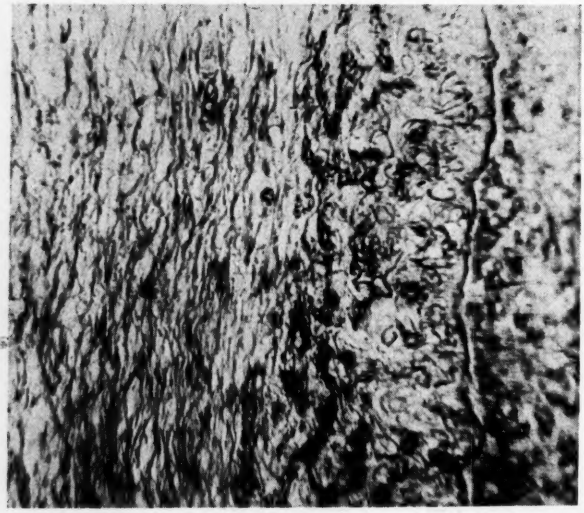

Fig. 26. — Fetus No. 31, CH length 25.0 cm. Longitudinal section of the right ureter. Gomori's silver impregnation. 5 μ. × 280. The basement membrane is distinctly seen. Coiled, thick fibres are seen below the membrane and longitudinal, parallel fibres in the periphery.

but the cells near the basement membrane frequently have a cubical shape.

Mesenchymal Cells. — These cells, which belong chiefly to the middle layer of the cellular wall structure, are rather small and oval, and the large, deeply stained nucleus leaves space for only a small band of cytoplasm. Thus they differ clearly from the large, light-coloured epithelial cells and from the elongated muscle cells, and they form close groups in the immediate vicinity of the basement membrane, becoming more sparse towards the periphery.

Basement Membrane. — The basement membrane, which closely participates in the growth events, is brought out also in the fifth month by different staining methods as a distinct border layer between the connective tissue and the epithelium (Fig. 26).

Reticular and Collagenous Fibres. — In the subepithelial layer in particular, these connective tissue fibres become markedly thickened and deeper in tone, at the same time as they form in several places parallel, wavy fibre bundles. In the area of muscular tissue the fibres are thinner and lighter in colour, but in the periphery they again collect into a close network of chiefly longitudinal,

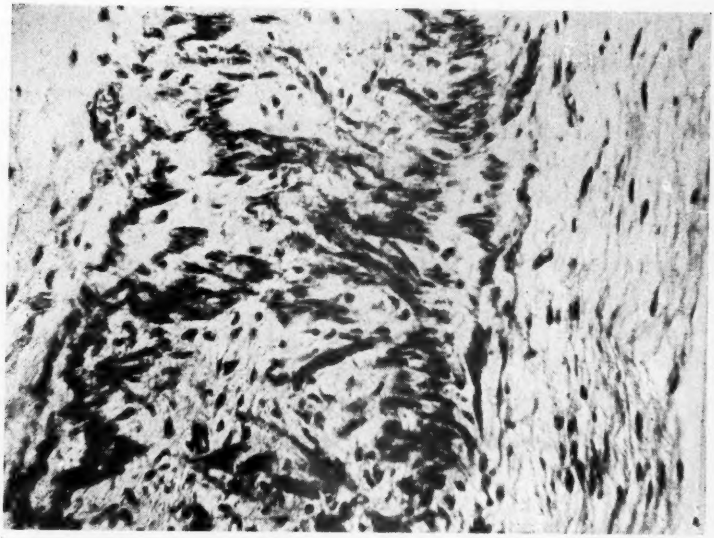

Fig. 27. — Fetus No. 31, CH length 25.0 cm. Longitudinal section of the right ureter. Kornhauser's staining. 5 μ. $\times$ 280. Strong, circular portions of muscle spirals begin to turn longitudinally on each side. Light-coloured elastic network is seen here and there between the muscle cells. In the periphery there are wavy collagenous fibres and capillaries.

parallel fibres (Fig. 26), which forms a sharp margin against the surrounding loose connective tissue containing sparse fibres.

The reticular fibres, which are situated at various levels, differ greatly in form. The upper, most intensely staining fibres are thick, very wavy or spiral and frequently send out delicate processes, while straight, fine and light-coloured fibres form a lower layer. The latter fibres are similar in type to the fine reticular fibres of the netlike connective tissue sheath of the muscle cells.

The strong subepithelial collagenous fibres, morphologically resembling reticular fibres, are reduced in width, as they spirally encircle the wall, towards the adventitial, longitudinal portion of the fibre layer.

Elastic Fibres. — In similarity to the other types of connective tissue fibres, the elastic fibres mature very slowly in form and tinctorial properties (Hass 1939), but as the dense wall layer increases in thickness its elastic fibre network also gradually becomes stronger, surrounding the muscle cells in particular (Fig. 27).

Together with the entire connective tissue, the elastic fibres extend to near the basement membrane, continuing along the wall of the calyces into the fornix. In the papillae the basement membranes of the collecting tubes, brought out clearly also with elastic stains, stand out distinctly from the surrounding tissue, which otherwise contains only reticular and collagenous fibres.

Morphologically the elastic fibres are very uniform in thickness and wavy. Frequently a number of subepithelial fibres lie closely parallel.

Muscle Fibres. — The fifth month is also a period of marked development of the muscular tissue. The muscle fibres, which are thin up to quite near the fornix, gradually become thicker towards the renal pelvis and the ureter, especially where the fibres are arranged in circular formation. However, no proper anatomic sphincter muscles (*e.g.*, the muscle of Henle, 1862, or *m. sphincter papillae, m. sphincter calycis, m. sphincter pelvis*) are visible, and the muscular tissue is a structural entity, which nevertheless may physiologically be divided into sections according to the local functional demands of the different areas of the urinary excretory passages.

The arrangement of the muscle fibres is very clear and it is possible to follow in the specimens the gradual reduction of the angle of elevation of the longitudinal, unbranched fibres in the peripheral portions of the connective tissue, at the same time as they become thicker and unite into strong fibre bundles, which frequently form branches. As they rise from the horizontal plane, these fibres (in the sections) seem at first short and then again longer, and they are thinner as they approach the subepithelial connective tissue layer. In the renal pelvis the longitudinal muscle fibre spirals are very numerous, while the circular plexus network characterises the muscular tissue of the ureter (Fig. 27).

The multinuclear muscle cells vary somewhat in form according to their situation. In the area of circular muscular tissue the long, narrow cells with oval nuclei are greatly broadened and the nucleus, which is rich in chromonemic filaments and is surrounded by a deeply staining cytoplasm, is rounded out.

Blood Vessels. — The capillaries, which supply all parts of the wall, are most numerous in the area of muscle fibres and in the subepithelial layer of connective tissue.

Fig. 28. — Fetus No. 24, CH length 18.5 cm. Longitudinal section of an artery in the right renal pelvis. Synthetic orcein-fast red. 50 μ. $\times$ 280. Longitudinal folds of internal elastic membrane are well demonstrated.

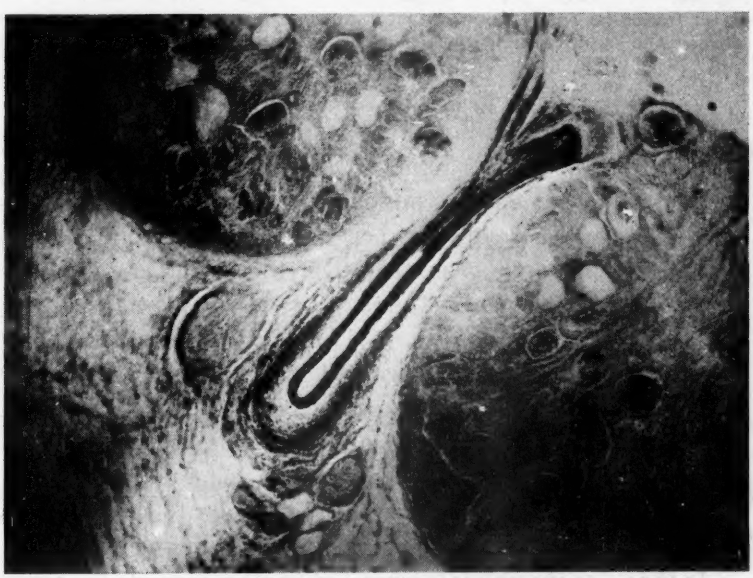

Fig. 29. — Fetus No. 27, CH length 22.0 cm. Longitudinal section of the right kidney. Synthetic orcein. 50 μ. $\times$ 90. The arrangement of elastic tissue in an artery of the renal pelvis is clearly visible.

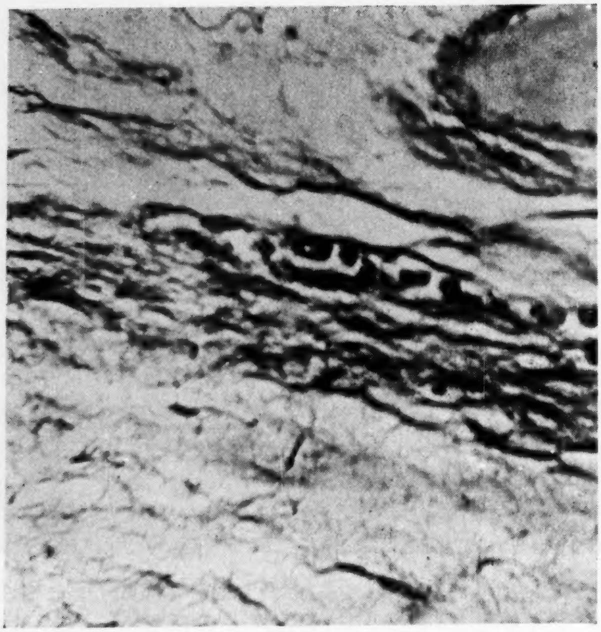

Fig. 30. — Fetus No. 30, CH length 24.3 cm. Longitudinal section of the right ureter (outer layer). Silver carbonate staining. 5 μ. $\times$ 700. Nerve fibres are seen on the outer margin of the longitudinal muscular layer, adjoining the wall of a capillary.

The tunica adventitia of the arteries contains a large number of thick, deeply staining reticular and collagenous fibres, and the external elastic membrane sends out into the tunica media numerous dark fibres, which frequently penetrate the latter and terminate in the internal elastic membrane (Dürck). As earlier, both elastic membranes can be demonstrated very clearly by elastic staining and silver impregnation (Fig. 28). Along with general growth, the elastic fibres outside the external elastic membrane have become thicker and their network has expanded (Fig. 29).

Practically all that is seen of the muscle cells of the tunica media between the elastic membranes in longitudinal sections is their large, oval nuclei, which are perpendicular to the membranes, indicating the mainly circular direction of the muscle fibres.

In the veins the wall is thicker, but in other respects the arrangement of connective tissue fibres is similar to that in the preceding month. The internal elastic membrane is seen clearly here and there

only, and thus its occurrence is greatly variable, probably due to the uneven structure. The mostly circular muscle cells are very fine in thickness and few in number.

Nerve Fibres. — As the muscular tissue is already at this stage very strong, numerous fine nerve fibres can be seen in it, in addition to those terminating in the blood vessels and freely in the connective tissue. In longitudinal sections of the ureter, groups of very long nerve fibres are frequently seen in the periphery, sometimes adjacent to the thin wall of the vessels (Fig. 30).

In the kidney parenchyma proper, the nerve fibres in some places run very clearly in the immediate vicinity of the veins; this has also been observed in animal specimens (mouse) in block impregnation, according to Nonidez (1939).

No ganglia are visible in the wall, nor can any myelin sheaths be demonstrated with osmium tetroxide in the renal pelvis and the ureter of a fetus 24.3 cm long (CH) (No. 30).

SIXTH INTRAUTERINE MONTH

Fetuses No. 32—35, CH Length 26.3—32.5 cm

Epithelial Cells. — The epithelium, which very rapidly became transitional in type during the preceding months, grows somewhat stronger in the sixth month.

The cytoplasm in the oval, flat cover cells is here and there more dense and is shown by different stains to be an apical, dark, membranous protection for the epithelium (Fig. 31). The cells immediately under them are at first round or cylindrical, with pale cytoplasm and a large, round, centrally or apically situated nucleus. The cells become smaller and cubical in the basal direction towards the basement membrane. They are arranged, when the organ is contracted, in three to five layers and are, on the whole, larger and more elongated in the ureter than in the renal pelvis and calyces. The epithelium of the collecting tube consists of tall, narrow cells, with dark cytoplasm surrounding the basally situated nucleus.

Mesenchymal Cells. — The observations in the connective tissue in the sixth month are similar to those made in the fourth and fifth

months in regard to the form, staining and situation of the small mesenchymal cells and of the large, fibroblast-type cells containing a large amount of pale cytoplasm. No marked changes occur in these cells during the sixth fetal month.

Basement Membrane. — Due, in part at least, to the strengthening of the subepithelial connective tissue layer, the basement membrane no longer forms, as in the first stages of development, a sharply defined, continuous boundary layer in the preparations treated with collagen stains or silver nitrate complexes (Fig. 32), but it is still distinctly brought out with resorcin-fuchsin. Nevertheless, the possibility also of structural submicroscopic changes in the prenatal formation of the basement membrane cannot be totally excluded.

Reticular and Collagenous Fibres. — Morphologically variable reticular and collagenous fibres, lying in various directions, stand out in the basic network of the wall, which is composed of fine, light-coloured and uniformly thick fibres.

In the periphery and in the narrow area adjoining the basement membrane they are arranged mainly in a longitudinal, parallel direction and take a spiral form when they approach the horizontal plane in muscular tissue and in the subepithelial connective tissue layer proper (Fig. 33). In the latter the fibres are very thick and coiled and frequently form wavy bundles. Within the muscular tissue the fibres are interlaced, and metal impregnation frequently brings out a silver sheath of fine connective tissue fibres, which surrounds the muscle cells. In view of the spiral form of the fibres, the boundaries between the various parts are not abrupt, especially in the renal pelvis, and transition from one layer to another is gradual (Fig. 32).

Elastic Fibres. — Except for structural strengthening extending from the basement membrane to the periphery of the elastic fibre network of the wall, the sixth month brings no essential morphological or tinctorial changes in the individual fibres and their arrangement described in connection with the fifth month.

Muscle Fibres. — In the renal pelvis, and especially in the ureter, the muscular tissue, which is closely surrounded by connective tissue fibres, is rather strong at the end of the sixth intrauterine month (Fig. 31). The spiral muscle fibres of the ureter are joined together at their middle parts, forming thick, branched,

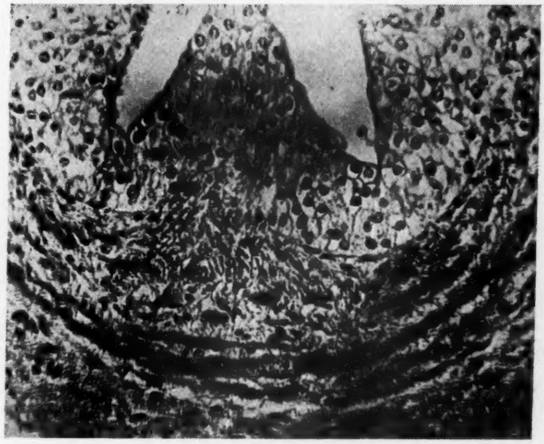

Fig. 31. — Fetus No. 32, CH length 26.3 cm. Cross-section of the left ureter. Kornhauser's staining. 5 μ. $\times$ 280. There are strong circular and thin longitudinal muscle fibres. The elastic fibres are slender and light-coloured. A close network of collagenous fibres is visible in the periphery. Cytoplasmic condensation in cover cells is seen here and there.

Fig. 32. — Fetus No. 32, CH length 26.3 cm. Cross-section of the right ureter. Gomori's silver impregnation. 5 μ. $\times$ 280. There are strong, wavy fibres near the epithelium, with sharp margin towards the loose connective tissue. Fibre sheaths are seen around muscle cells. The basement membrane is not seen distinctly.

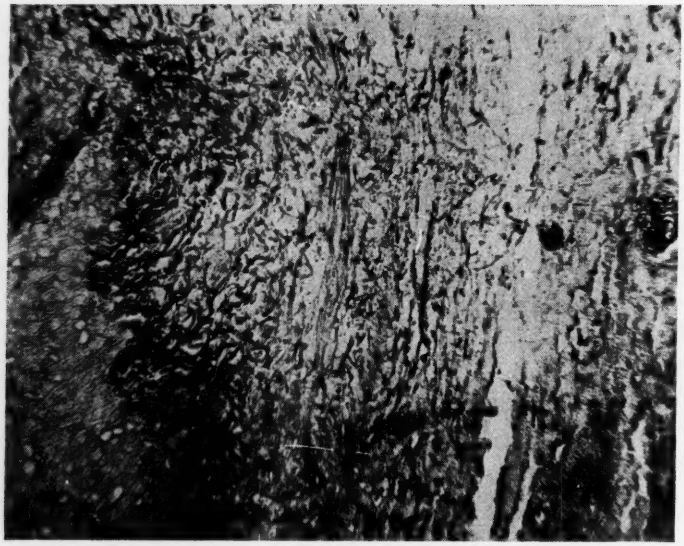

Fig. 33. — Fetus No. 34, CH length 30.2 cm. Diagonal section of the left renal pelvis. di Maggio's staining. 5 μ. $\times$ 280. A strong reaction is noted in the subepithelial reticular fibres.

cord-like clusters, but even now no special thickening resembling a sphincter muscle can be observed at the junction of the renal pelvis and the ureter. In following their increasing angle of elevation towards the cavity, it is noted that the muscle fibres become finer and change into longitudinal, slightly branched cells containing oval nuclei and continuing fairly deep into the subepithelial connective tissue layer, whose folds into the interior of the cavity they follow closely. Morphologically they resemble the sparse fibres in the periphery, which also are longitudinal.

Blood Vessels. — In addition to a strengthening of the spiral connective tissue fibres in the tunica adventitia of the arteries, the muscle cells, which mostly appear circular in the cross-sections, increase in length and their large, dark nuclei can be very well distinguished in some places (Fig. 34). No notable changes from the picture seen in the preceding month are observed in the elastic membranes.

The muscle cells in the venous wall are also strengthened but remain much weaker than those in the arterial wall. There are a large number of elastic fibres, but no continuous internal elastic membrane is discernible even now, there only being next to the

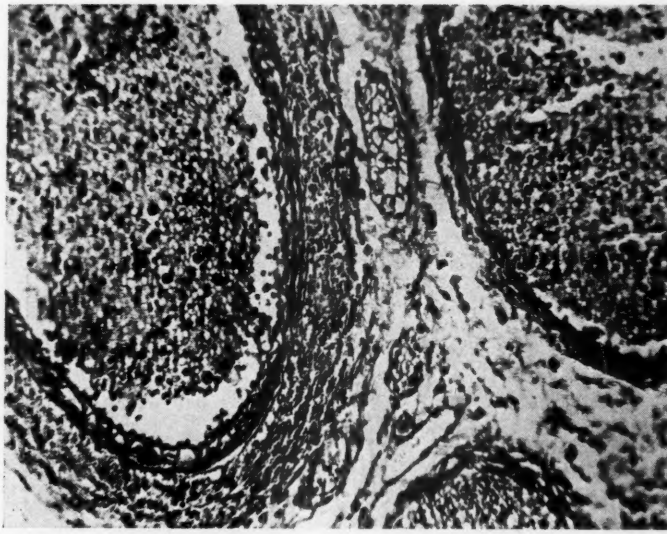

Fig. 34. — Fetus No. 33, CH length 27.0 cm. Cross-section of an artery and vein in the left renal pelvis. Masson's staining. 5 μ. $\times$ 280. Circular muscle fibres are seen in some places. Note the thickness of the walls.

endothelial tissue elastic fibres which frequently can be observed for long distances as a thin membrane (Fig. 36).

Nerve Fibres. — The subepithelial nerve fibre plexus shows growth and increase in thickness, but the arrangement of the nerve fibres in other respects is similar to that described in the preceding months, for instance in the blood vessel walls (Fig. 35). Demonstration of myelin sheaths by osmium tetroxide was not successful even now in the case of the renal pelvis of a fetus 32.5 cm long (CH) (No. 35).

SEVENTH INTRAUTERINE MONTH

Fetuses No. 36—37, CH Length 34.0—35.5 cm

If it were possible to give a fully exact representation, for instance in the form of a graph, of the different changes in the development of the tissues of the renal pelvis and the ureter as seen under the microscope, such a graph would show a curve that rises relatively abruptly up to about the middle of intrauterine life

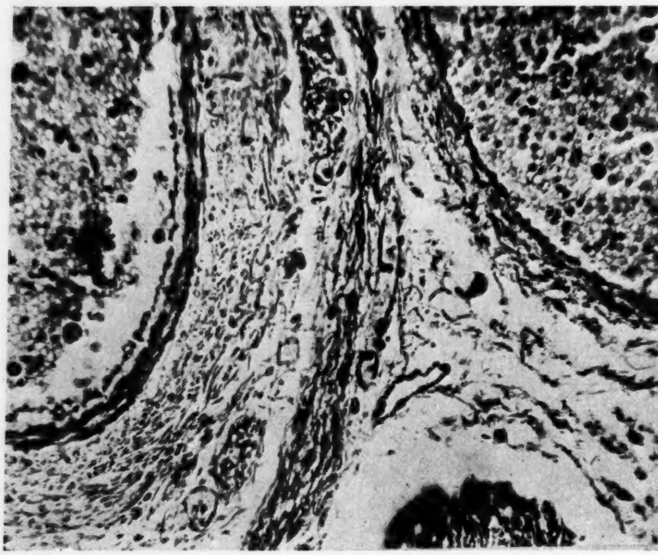

Fig. 35. — Fetus No. 33, CH length 27.0 cm. Cross-section of the left kidney. Ungewitter's staining. 5 μ. $\times$ 280. Nerve fibres of various thicknesses are seen between an artery and vein of the renal pelvis.

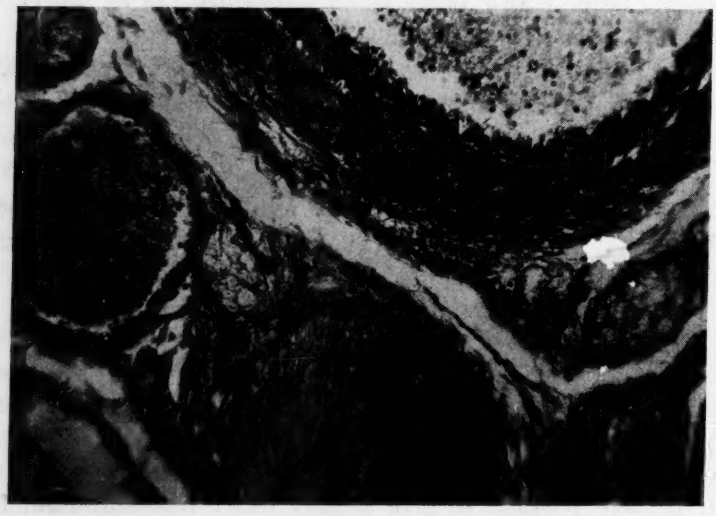

Fig. 36. — Fetus No. 33, CH length 27.0 cm. Cross-section of a nerve, artery and vein in the right renal pelvis. Resorcin-fuchsin. 20 μ. $\times$ 280. Dark elastic fibres are seen in the epineurium. Outside the external elastic membrane there are a large number of elastic fibres, seen as dots, and some strong fibres extending from the membrane to the tunica media. The elastic membrane of the vein is visible in some places.

but then maintains a nearly horizontal level, especially in the last stages before term. The events during the last three months are, in fact, slight in the specimens examined.

Throughout intrauterine life, no noteworthy difference was observed between the right and the left renal pelves and ureters or between the two sexes in the specimens examined.

Epithelial Cells. — Epithelial folds due to cell proliferation are seen especially in the ureter, whereas the basement membrane and the subepithelial connective tissue follow the folds only slightly. The membrane-like condensation of cytoplasm in the cover cells is seen in some places very clearly as a dark band, for instance following the reduction of silver nitrate complexes.

Basement Membrane. — No notable changes occur in the basement membrane during the seventh month.

Reticular and Collagenous Fibres. — The specimens treated with silver nitrates or with highly acid sulphonated triphenylmethane dyes show distinctly a delicate basic network which extends through the thickness of the wall and which consists, in the one case, of dark and, in the other case, of light blue fibres. These fibres are in some cases observed to arise as processes from mesenchymal cells. In addition to this, the reticular and collagenous fibres have the same form and arrangement as has been described previously, but they are especially strong in the subepithelial connective tissue layer (Fig. 37).

Elastic Fibres. — In the connective tissue adjoining the basement membrane and in the area of muscle fibres, the close network of uniformly thick elastic fibres supports the wall in the manner described earlier.

Muscle Fibres. — No notable strengthening of the longitudinal muscle fibres or of the fibre bundles representative of the spiral circular muscular tissue is seen this month in the muscular tissue of the renal pelvis and the ureter (Fig. 38).

Blood Vessels. — Only the dark nuclei of the numerous muscle cells of the arterial wall are seen in the longitudinal sections, being well brought out as closely set vertical pillars between the elastic membranes. In other respects the arrangement and thickness of tissues in the arteries and veins remains practically unchanged in the seventh month (Fig. 39).

The abundant network of capillaries extending from the ad-

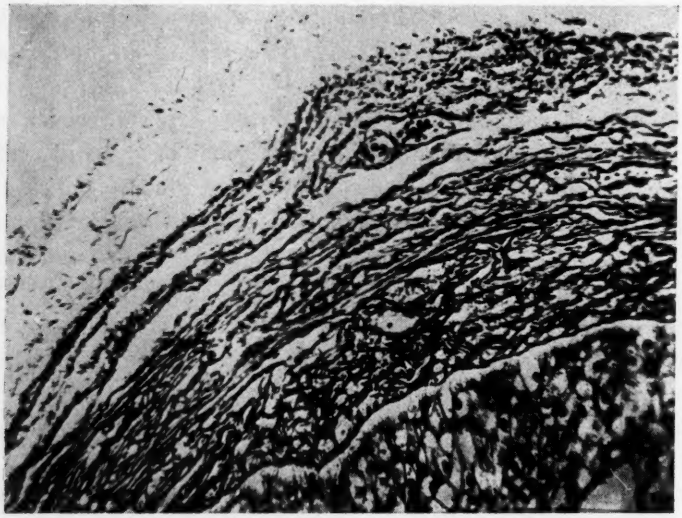

Fig. 37. — Fetus No. 36, CH length 34.0 cm. Cross-section of the left ureter. Gomori's silver impregnation. 5 μ. $\times$ 280. The reticular fibres become stronger as they approach the basement membrane, which is occasionally discernible.

Fig. 38. — Fetus No. 37, CH length 35.5 cm. Cross-section of the left ureter. Kornhauser's staining. 5 μ. $\times$ 90. General arrangement of connective tissue and muscular tissue in the wall.

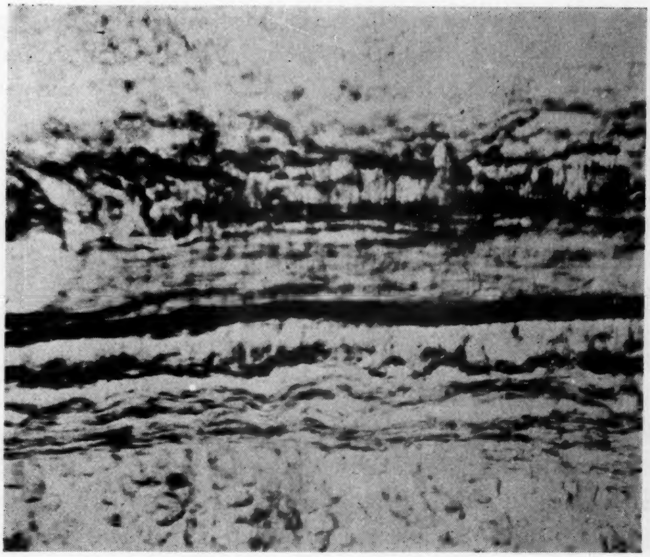

Fig. 39. — Fetus No. 36, CH length 34.0 cm. Longitudinal section of an artery and vein in the left renal pelvis. Resorcin-fuchsin. 20 μ. $\times$ 890. The internal elastic membrane is very strong, and the external elastic membrane is also fairly thick. A large number of dark elastic fibres is seen in the venous wall.

ventitia to the basement membrane of the renal pelvis and the ureter again attacts attention. The fairly large capillaries in the periphery have a tortuous course in the muscular tissue towards the epithelium and terminate as small blood vessels under the basement membrane.

Nerve Fibres. — At this stage, silver impregnation reveals no marked changes in the unmyelinated axis cylinders as compared with the observations made in the sixth month.

EIGHTH INTRAUTERINE MONTH

Fetuses No. 38—39,
CH Length 38.0—43.0 cm

Epithelial Cells. — In the specimens of the eighth month, epithelial proliferation is fairly slight and the cells in the transitional epithelium are variably arranged in two, three or more layers, depending on the situation of the cells and the state of contraction

of the organ. The cover cells, which are very numerous in the ureter but occur in the renal pelvis in few places only, stand out distinctly from other epithelial cells, which are large and pale in colour.

Mesenchymal Cells. — The small mesenchymal cells, together with the rather large cells of fibroblast type, continue to form in the seventh and eighth months the framework of the cellular structure of connective tissue and are most abundant in the dense portion of the wall.

Basement Membrane. — When the renal pelvis and ureter specimens are examined from the point of structural density, the basement membrane may be considered the termination of the increasing condensation of tissue, which starts at the loose connective tissue in the periphery and becomes more and more clearly fibrous in structure. With the growth of the wall, however, the basement membrane begins to lose its earlier distinctness in several places and becomes merged in the strong connective tissue layer. In such places it can no longer be clearly distinguished.

Reticular and Collagenous Fibres. — Together with the elastic fibres, these strong spiral fibres and wavy fibre bundles serve, in the form of a close network, as the actual supporting framework of the wall from the fornix downwards. In silver impregnation the muscle fibres are seen to be surrounded by the sheaths of fine connective tissue fibres described above, which follow their spiral course.

Elastic Fibres. — The morphology, arrangement and staining properties of the elastic fibres are, on the whole, almost unchanged during the eighth month, although some individual fibres may already be observed to be thicker and darker.

Muscle Fibres. — In the muscle fibres there is moderate strengthening, especially in the longitudinal portions of the spirals. These portions are also frequently discerned distinctly in the periphery (Fig. 40). The fibres adjacent to the subepithelial connective tissue layer extend very near the epithelium in some places. In the folds of the ureter, however, they do not extend beyond the groove of the folds.

Blood Vessels. — The outermost elastic fibres of the arteries have increased in size and thickness and they closely adjoin the external elastic membrane. Thus a very strong fibrous elastic

Fig. 40. — Fetus No. 38, CH length 38.0 cm. Longitudinal section of the right renal pelvis. Kornhauser's staining. 5 μ. $\times$ 280. The basement membrane is visible here and there. Longitudinal muscle fibres and arterial structure are distinct.

network is formed in the tunica adventitia as a response to external influences. In some places it almost obtains the strength of the internal elastic membrane. Evidently increasing functional demands have been a contributing factor in the development of this structural arrangement.

The venous walls undergo a general, somewhat variable thickening. The capillary network shows no changes deviating from what has been described previously.

Nerve Fibres. — A large number of slender nerve fibres accumulate in many places in the loose connective tissue outside the muscular layer, forming a plexus from which some fibres continue into the muscular tissue.

FROM THE NINTH INTRAUTERINE MONTH TO BIRTH

Fetuses No. 40—41,
CH Length 48.5—50.5 cm

Epithelial Cells. — In accordance with the early maturation changes in the cellular wall of the renal pelvis and the ureter, the final stages of development up to birth bring out no new features even in the epithelium, and its different cell types continue to serve as a protection for all types of tissue. Noteworthy is the dark, apical band of cytoplasm in the cover cells, which is frequently seen very clearly, for instance after the adsorption of resorcin-fuchsin, when the epithelial cells proper lie between two membrane-like structures.

Mesenchymal Cells. — The small mesenchymal cells with round nuclei, which from the very beginning have taken an active part in the development, are also now abundant in all parts of the connective tissue of the wall but are in particular accumulated in the layer below the basement membrane. The larger, often branched cells of the fibroblast group, which are rich in cytoplasm, are frequently seen in the preparations.

Basement Membrane. — With the gradual growth of the subepithelial tissue and thickening of its fibres, the basement membrane seems to merge more closely with its surroundings, even though in the new-born it is still brought out here and there by silver impregnation and other methods of staining. It is possible that the basement membrane itself and the adjacent fibres become stronger along with the general development of the kidney, thus preparing for flexible action and for adjustment to the changing postnatal functional states of the renal pelvis and the ureter.

Reticular and Collagenous Fibres. — The reticular and collagenous fibres and fibre bundles have by the end of fetal life attained a fairly great thickness, especially in the subepithelial layer (Fig. 41). However, their tinctorial changes are relatively small. A very marked difference can nevertheless be seen in stainability on comparison of the silver impregnated specimens from the oldest fetuses in this series and similar specimens from the adult renal pelvis. In silver impregnation the black, coiled fibres of the former have in the corresponding situations in the latter become light brown in colour and wavy in shape.

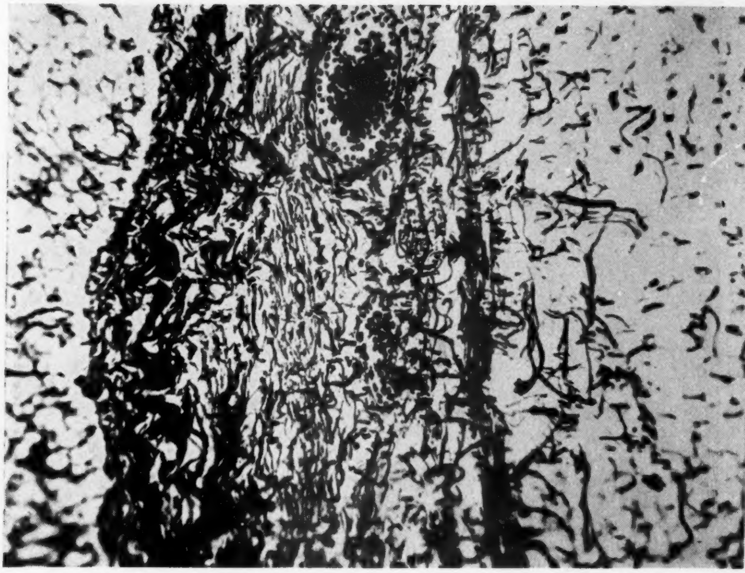

Fig. 41. — Fetus No. 41, CH length 50.5 cm. Diagonal section of the right ureter. Gomori's silver impregnation. 5 μ. $\times$ 280. There are thick, dark reticular fibres, which here and there are wavy.

The parallel, slender fibres in the periphery become stronger as they curve towards the lumen of the renal pelvis and the ureter in the muscular tissue and in the section of connective tissue adjoining the epithelium. However, they frequently become thinner again in a very narrow zone under the basement membrane, at the same time as they turn lengthwise, so that the basement membrane is also clearly distinguished.

Elastic Fibres. — In the peripheral portion of the dense network of fine fibres, which is brought out in thick specimens stained by the usual elastic staining methods, numerous individual fibres are observed to become stronger and much darker in colour during the last fetal month (Fig. 42). These fibres, which are distinctly dark in this period for the first time, are now morphologically similar to the elastic fibres of the adult renal pelvis, being no longer regarded as the preliminary stage of development of elastic tissue.

Muscle Fibres. — Along with the total development of muscular tissue, also the longitudinal parts of the muscle fibres stand out with increasing clarity in the last stages of fetal life. The first strong

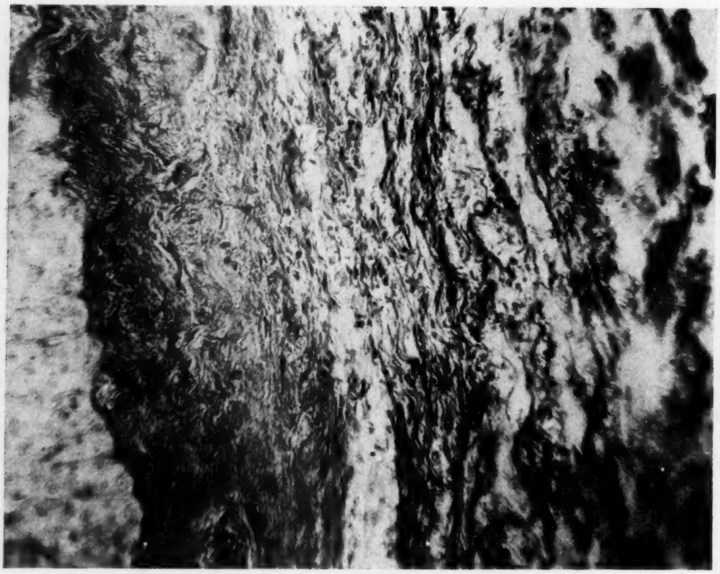

Fig. 42. — Fetus No. 40, CH length 48.5 cm. Cross-section of the left ureter. Resorcin-fuchsin. 20 μ. $\times$ 280. Strong, dark elastic fibres are seen in the wall. The basement membrane is not clear.

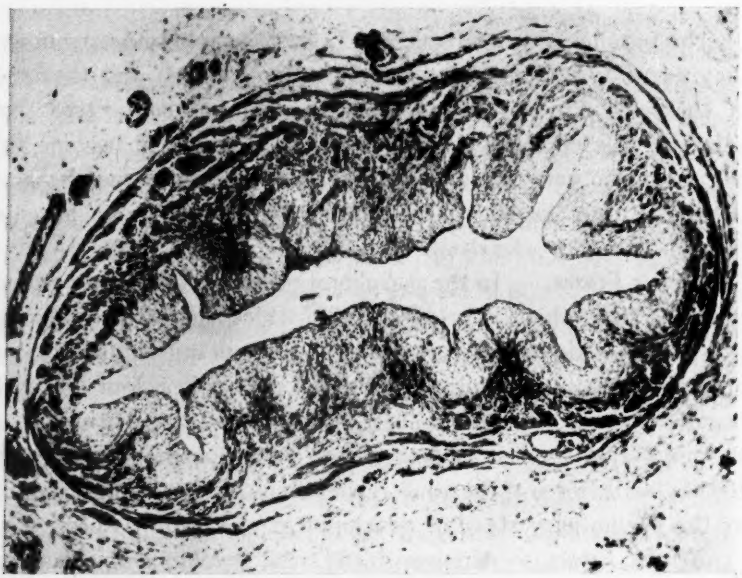

Fig. 43. — Fetus No. 41, CH length 50.5 cm. Diagonal section of the right ureter. Hematoxylin-orcein-picroindigo carmine. 10 μ. $\times$ 90. General view of the strong muscular tissue and the subepithelial connective tissue. Note the quantity of the capillaries.

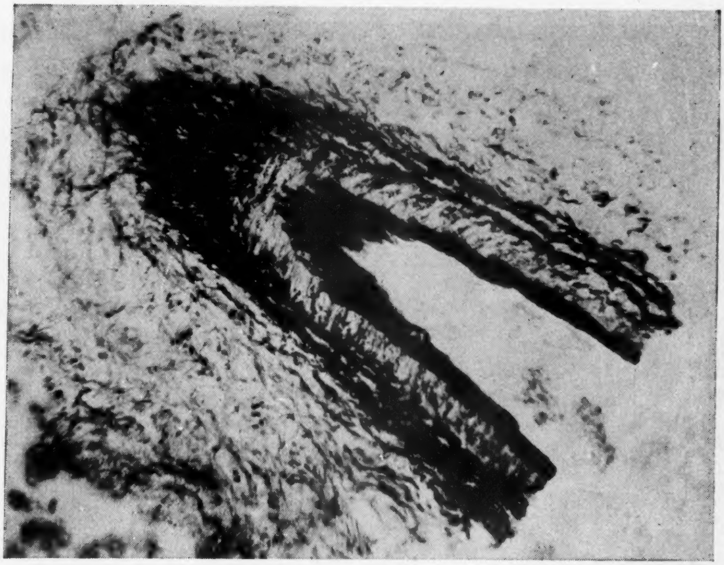

Fig. 44. — Fetus No. 40, CH length 48.5 cm. Longitudinal section of an artery in the left ureter. Resorcin-fuchsin. 20 μ. $\times$ 280. The elastic membranes are very strong. Circular elastic fibres are seen in the tunica media.

muscle fibres representative of the circular muscular tissue have thus grown towards both the periphery and the subepithelial connective tissue layer, and they have at the same time taken a nearly spiral course. In the periphery the muscle fibres are fairly sparse, as they are dispersed over a larger area than those near the centre. The contribution of these fibres, in particular, in the structure of the pelvic and ureteral wall becomes more evident and notable during the period in question (Fig. 43).

Blood Vessels. — The elastic membranes of the arteries are very thick and especially the external elastic membrane and the adventitial elastic fibres attached thereto have become very much stronger (Fig. 44). The muscle cells in the tunica media have also increased in size and numerous spiral, dark elastic fibres coming from the external elastic membrane are seen in it. No notable changes have occurred in the reticular and collagenous fibres in the tunica adventitia.

It is not possible to distinctly distinguish different coats in the venous walls, and they have a dense netlike structure. The endo-

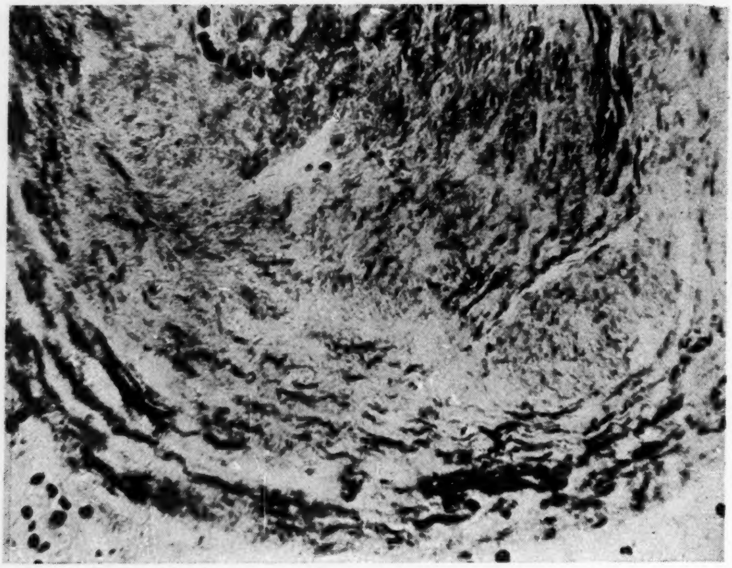

Fig. 45. — Fetus No. 40, CH length 48.5 cm. Cross-section of the left ureter. Romanes's staining (1946). 5 μ. × 280. Most of the nerve fibres are very dark; they are slender in the area of muscular tissue and thick in the adventitia.

thelial cells are an elongated oval in shape, as in the arteries, and the internal elastic membrane is distinct and wavy in some places in the cross-sections but it still is very thin in comparison with, for instance, the arterial internal elastic membrane. As the wall, in which elastic fibres are abundant, becomes stronger, the muscular cells also are more pronounced within the network of connective tissue fibres.

The thin network forming the wall of the capillaries and bordering on the endothelial tissue is built up of all different types of connective tissue fibres, and it is seen also in the capillaries which are quite adjacent to the basement membrane. Their tortuous course in the area of the muscle fibres and in the subepithelial connective tissue layer can be followed clearly, for instance by means of the bright red erythrocytes in orange G staining.

Nerve Fibres. — The course of the development of the nerve fibres in the wall of the renal pelvis and the ureter towards their definitive arrangement, which was observed already during the eighth intrauterine month, becomes still more distinct at the end

of fetal life. In the area of the muscle cells there are a large number of fine nerve fibres, which appear to have their origin in the plexus situated in the adventitial connective tissue of the wall. This plexus is now stronger than in the preceding month and its fibres are stained rather dark by impregnation (Fig. 45). After passing through the muscular layer the fibres unite, as described earlier, to form a narrow plexus under the basement membrane. The close relation of the nerve fibres to the capillaries in the wall is also seen clearly in some places in the specimens examined.

DISCUSSION

A. PRENATAL HISTOGENESIS OF THE RENAL PELVIS AND THE URETER

Of the numerous closely related and influential structural parts and factors whose intimate and harmonious interaction during intrauterine life promotes the developmental processes evenly and gradually towards the definitive entity of the organism, the present investigation is concerned with the appearance and organisation of the histological structural components of the wall of the renal pelvis and the ureter. Already at a very early stage, during the first trimester of intrauterine life, a tissue architecture best suited to the functional purpose and the structural mechanism of the renal pelvis is seen in the mesodermal tissue which surrounds the epithelium. The initial arrangement of the cells and fibres in this tissue indicates that the developmental processes proceed from the subepithelial tissue towards the periphery. This may take place independently in the different tissues but it is also influenced greatly by environmental factors.

At about the middle of the third intrauterine month the epithelium changes rapidly here and there from simple, sometimes pseudostratified type to the transitional type, as was also observed by Felix. The observation may be made that in areas where the epithelium is well developed the other layers of the wall are also advanced.

In the surrounding tissues the most active growth occurs, at least during the first months of intrauterine life, in the immediate vicinity of the epithelium. The fibrous basement membrane, which chiefly might be of connective tissue origin, intimately takes part in this growth. Already at the end of the second month, dense accumulations of mesenchymal cells are seen around the basement

membrane of the renal pelvis, as was also demonstrated by Jánošík and Ludwig (1950/51). In the ureter these cells are arranged in a similar manner somewhat earlier. Epithelial cell proliferation at the junctions of the ureter and the pelvis and of the pelvis and the calyces has a notable influence on the development of both the epithelium and the connective tissue, as the cells and fibres in the proliferative areas greatly increase in number and orientate themselves vertically to the basement membrane, in the direction of the exerted pressure. The continuity of the basement membrane is destroyed at the same time. However, the epithelial framework as a whole also increases in length, and along with the strengthening of the structure of the wall as a result of condensation, the spiral fibre architecture is brought out with increasing clarity. When the longitudinal fibres of the periphery approach the centre, their angle of elevation diminishes as their direction becomes more circular. The fibres revert to their original direction in the narrow zone under the basement membrane. Due to thickening of the fibres and to possible structural changes in the basement membrane, the latter, which in the early stage was a distinct line of demarcation between the epithelium and the connective tissue, merges more and more with its surroundings towards the end of fetal life.

The anlages of muscular tissue, *i.e.*, the muscle fibres, appear in the vicinity of the epithelium in the form of large cells with oval nuclei. As they develop, they become longer and stronger, especially during the second trimester, and recede at the same time in the peripheral direction. In the wall of the renal pelvis the spiral fibres thus formed lie in various directions and are weaker than in the wall of the ureter. This musculature is anatomically uniform and cannot be clearly divided into separate layers, since, for instance in the ureter, the longitudinal fibres of the periphery, at the same time as they thicken, gradually turn in their middle portions in a circular, spiral direction. Thereafter, their angle of elevation in the subepithelial connective tissue again increases. No specialisation pointing to independent sphincter muscles can be discerned.

It seems probable that this wall structure which develops during intrauterine life reflects both the form of the organ and its adaptation towards future functions. The fibres in the thin wall of the renal pelvis, which latter has a large cavity and serves chiefly as a reservoir regulating the flow of urine, lie mainly in a

longitudinal direction and the epithelium is thrown into folds only in the areas of proliferation. In the ureter, which actively conveys the urine, the fibre arrangement is clear, and strong circular connective tissue fibres and muscle fibres are an important component in the network of the wall, and the epithelium, with the underlying connective tissue, forms already in the fourth month an abundance of folds when in the contracted state.

Whether the connective tissue fibres develop directly from the mesenchymal cells (Stearns 1940) or indirectly from the ground substance (Weiss 1929), they are in any case products of the growth and specialisation of the same primitive fibre, as observed also by Merkel and Krauspe. These primitive fibres, which are brought out by several dissimilar staining methods, form a fine network in the wall of the renal pelvis and the ureter. This network is present through intrauterine life, and the connective tissue fibres in the different layers probably mature gradually from the network by means of slow morphological and chemicophysical changes.

The development thereafter appears to take place in two different directions. On the one hand are formed the reticular and collagenous fibres, which, although of a different thickness, are representatives of the same type of fibre (Dettmer *et al.* 1951, v. Herrath and Dettmer 1951), and on the other hand the elastic fibres. By the end of fetal life the fibres that reduce silver salt complexes have attained a moderately great thickness, although they still are rather dark in colour, and they are also brought out in the same form and numbers with various collagen stains. This points to the slow maturing of the fibres in this group and to their close relationship even at term.

On the other hand there is the appearance and slow change of the elastic fibres. In elastic staining the fibres of the wall itself, which at first are light in colour, appear darker and stronger only during the last intrauterine month, thus indicating the maturation that continues for some time postnatally.

In the architecture of the wall of the blood vessels, connective tissue fibres are predominant. Already in the very first stages of development a marked difference is noted between the elastic tissue in the blood vessels and that elsewhere in the renal pelvis and the ureter, probably as a result of early events of maturing. Both elastic membranes of the arteries take an intense stain and

appear to be strong already in the beginning of the third month. They rapidly increase in strength thereafter, thus showing the increasing adaptation of the arteries not only for pulse waves but also for the strain of the peristaltic movement of the renal pelvis and the ureter. The elastic fibres in the tunica adventitia and the tunica media stain very deeply and thus differ from other elastic fibres in the wall of the renal pelvis, which take a light stain by the same methods. The venous wall contains, in addition to other connective tissue fibres, a large number of dark, spiral elastic fibres, which interlace as a network and here and there form a very thin internal elastic membrane, present only in some places. The muscle fibres, which predominantly are circular, are at the end of intrauterine life moderately strong in the arteries but very weak and few in number in the veins.

The delicate capillary walls obtain support from the surrounding dense connective tissue network and especially from the elastic fibres, as is expedient with a view to their function and the continuous flow of blood.

As is the case with the above mentioned tissues, the third month plays an important part also in the appearance and development of nerve fibres. Both in the subepithelial connective tissue and in the immediate vicinity of the blood vessels of the wall there may be observed during this month some light-coloured, slender nerve fibres, such as later appear also in the muscular tissue as it becomes stronger. The fibres, which are unmyelinated, remain generally very light in tone in silver impregnation, thus, according to Nonidez, indicating their sympathetic postganglial origin. However, the differentiation of the nerve types by silver impregnation is very uncertain and the diverging observations of various authors are often due to different modifications in method. At the end of fetal life the fibres already stain fairly dark and form in the adventitial wall a plexus (De Muylder 1945) which sends out a large number of fibres to the muscular tissue and to the connective tissue underlying the basement membrane.

No noteworthy difference was observed between the right and left renal pelves and ureters or between the two sexes in the specimens examined.

B. COMPARISON WITH THE DEVELOPMENT OF THE URINARY BLADDER AND OTHER CAVITY ORGANS

According to the available results of investigations, the second and third intrauterine months, which are very significant for the development of the renal pelvis and the ureter, also play an important rôle in the formation and specialisation of the tissues in other cavity organs. There are certain exceptions to this, however, mainly depending on the situation and function of the organ, and the findings of different workers also vary somewhat. The comparison made below concerns the appearance of, chiefly, the muscular and elastic tissues in different organs, since these tissues occupy an important position in the structure and physiology of the organs.

In the urinary bladder, Felix observed that both the circular and the longitudinal muscle fibres develop during the second and third months, *i.e.*, approximately at the same time as muscular tissue appears in the renal pelvis. *M. sphincter vesicae internus* is seen in fetuses of 90 mm length.

It has also been possible to make comparisons with the development of the gastrointestinal tract and especially with the appearance of muscular tissue in its wall. According to the observations of Lewis (1911), Patzelt (1931) and Plenk (1932), the first circular muscle fibres may be discerned in the intestinal wall already during the second month, which is slightly earlier than is the case in the renal pelvis and the ureter. The third month sees the appearance of longitudinal muscle fibres to support the intestinal wall, whereas the *muscularis mucosae* does not develop until relatively late, in the fourth and fifth months.

The muscular tissue of the gall bladder and the sphincter of Oddi do not arise until the second and third months (Lee and Halpert 1932, Schwegler and Boyden 1937, Kirk 1948).

The formation of elastic tissue in the gastrointestinal tract has been found to occur much later than in the renal pelvis and the ureter according to my observations. In the intestinal wall this takes place during the sixth and seventh months (Patzelt, Plenk) and in the gall bladder only gradually after birth (Jeannin 1927).

On the other hand, elastic tissue is formed in the trachea and its branches and in the pulmonary arteries during the third month and towards the end of the fourth month, respectively(Grosser 1911, Setälä 1938).

This comparison of the development of muscular tissue in different organs shows, therefore, that the observations reported in the literature are, on the whole, concordant with each other

and with the findings made in this investigation, whereas differences, which sometimes may be great, are seen in the time of appearance of elastic tissue. It is very probable that the relation of the organs to their different functional demands already during the fetal stage is reflected in this arrangement, though histological observations alone on the early or late appearance of structural parts cannot fully explain the nature of the function of organs during fetal life.

C. SPHINCTER MUSCLES OF THE RENAL PELVIS

The junction of the renal pelvis and the ureter has been a feature of special interest in the upper urinary tract. Both normal and pathologic activity has been frequently observed at this junction, indicating the possible presence of a ring-like closing muscle, although it has not been possible to demonstrate a distinct sphincter muscle proper (Legueu, Fey and Palazzoli 1927, Hortolomei, Burghele and Streja 1937, Jewett 1940, Woodside 1948). In fact, Allemann (1935) ascribes the etiology of small hydronephroses to a disturbed opening reflex (achalasia) in this sphincter muscle, which, in similarity to pyloric stenosis, for instance, can be surgically corrected. Attempts have also been made to separate the muscular tissue of the renal calyces into different parts, and histological, physiological and roentgenological differentiation of sphincter muscles at the junction of the renal pelvis and the calyx and in the fornix has been reported (Muschat 1926, Oeconomos 1937, Hennig 1937, Narath). It has therefore been interesting to observe whether or not the investigation here reported would support the opinion that such a sphincter muscle is present already during fetal life. As has become evident from the detailed descriptions in this report, the conclusion may be drawn from the present study that the muscular tissue of the renal pelvis is, during intrauterine life at least, a structural entity which nevertheless becomes readily adapted to the various functional demands by division into separate physiological units, but in which no independent anatomic sphincter muscles can be discerned.

SUMMARY

In the reported investigation the histogenesis of the renal pelvis and the upper portion of the ureter was observed in human embryos and fetuses from the second intrauterine month to term. It was found that the third month is very significant for the appearance and development of all tissue types in these organs. No noteworthy difference was observed between the right and left renal pelves and ureters or between the two sexes in the specimens examined.

The epithelium, which in the first stages of intrauterine life is simple and in some places pseudostratified, is changed during the third month to transitional epithelium, on which cover cells appear in the fourth month. The epithelium and other layers of the wall correspond in regard to the stage of development, *i.e.*, where the epithelium is well developed, other parts of the wall are also advanced. The most active reaction in the surrounding tissues occurs in the immediate vicinity of the epithelium. The areas of epithelial proliferation play an important part in the increase and arrangement of connective tissue. However, the reciprocal influences also of all the other tissues is indispensable for the attainment of a harmonious structure of the whole.

During the fourth month, transformation of the mesenchyme to loose connective tissue begins. At this stage there is among the small mesenchymal cells a number of rather large, spindle-shaped cells resembling fibroblasts.

In all the specimens in this series, from the first embryo onwards, the basement membrane forms here and there a sharp line of demarcation between the epithelium and the connective tissue, to which latter it might be closely related by origin and structure. It follows the course of cell proliferation of the epithelium, is present only in some places, and by the end of fetal life has merged more and more into the strong connective tissue layer.

The origin of the connective tissue fibres is the unbranched primitive fibres situated at different levels and demonstrated by various staining methods. From these primitive fibres are formed the reticular and collagenous fibres, on the one hand, and the elastic fibres, on the other hand. The reticular and collagenous fibres are closely related during fetal life, and no notable variations in form and number are seen between the two throughout intrauterine life in silver impregnation and collagen staining of the same specimens. These fibres are disposed longitudinally in the periphery and are here seen in cross-sections as dots or short lines. On nearing the epithelium they become thicker and curve towards the horizontal plane. In the basement membrane and its immediate vicinity they frequently revert to their original direction.

The fibres demonstrated by elastic stains in the wall proper are at first slender and light in colour, and only during the last month of fetal life they become somewhat stronger and deeper in tone. Such fibres are seen, from the third month onwards, in all parts of the wall but most densely in the subepithelial connective tissue and around muscle fibres and capillaries.

The development of muscle fibres begins in the first half of the third month from large oval cells situated under the basement membrane, which rapidly become thicker and longer and at the same time recede peripherally. They are spirally disposed in the wall, and in the ureter their horizontal portions are very strong. The muscular tissue of the renal pelvis remains rather weak and no anatomic sphincter muscles can be observed at the junctions of the different parts of the pelvis.

The capillaries, which become organised during intrauterine life into a dense network, are already present, although few in number, in the second intrauterine month. In addition to circular muscle fibres, connective tissue is an important component in the structure of the small arteries, which are lined with oval endothelial cells. The elastic tissue in the arterial walls takes an intense stain, and the two elastic membranes, which are thrown into longitudinal folds, are brought out already in the beginning of the third month and are very strong by the end of fetal life. Circular spiral fibres extend here and there into the tunica media from the net-like external elastic membrane, outside which there also are a large number of longitudinal elastic fibres in addition to the reticular

and collagenous fibres of the tunica adventitia. The walls of the veins are rather thin and contain, besides connective tissue fibres and muscle cells, dark, thin elastic fibres and here and there a narrow internal elastic membrane, which begins to develop in the latter half of the third month.

In the third intrauterine month the specimens examined show some light-coloured, slender, unmyelinated nerve fibres, which closely adjoin the blood vessel adventitia and also terminate freely in the connective tissue below the basement membrane. No ganglia are visible in the wall. With growth of the muscular tissue, numerous nerve fibres are also seen in its area, and at the end of fetal life there is in the peripheral portion of the wall a plexus which sends out fibres into the muscular layer. These continue into the narrow subepithelial plexus, in which no intraepithelial terminations are discernible. At the time of birth the nerve fibres are stronger and deeper in tone as a result of possible maturation changes.

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